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(19) **United States**(12) **Patent Application Publication**
Koster et al.(10) **Pub. No.: US 2003/0180749 A1**(43) **Pub. Date: Sep. 25, 2003**(54) **METHODS FOR GENERATING DATABASES
AND DATABASES FOR IDENTIFYING
POLYMORPHIC GENETIC MARKERS**(76) **Inventors: Hubert Koster, La Jolla, CA (US);
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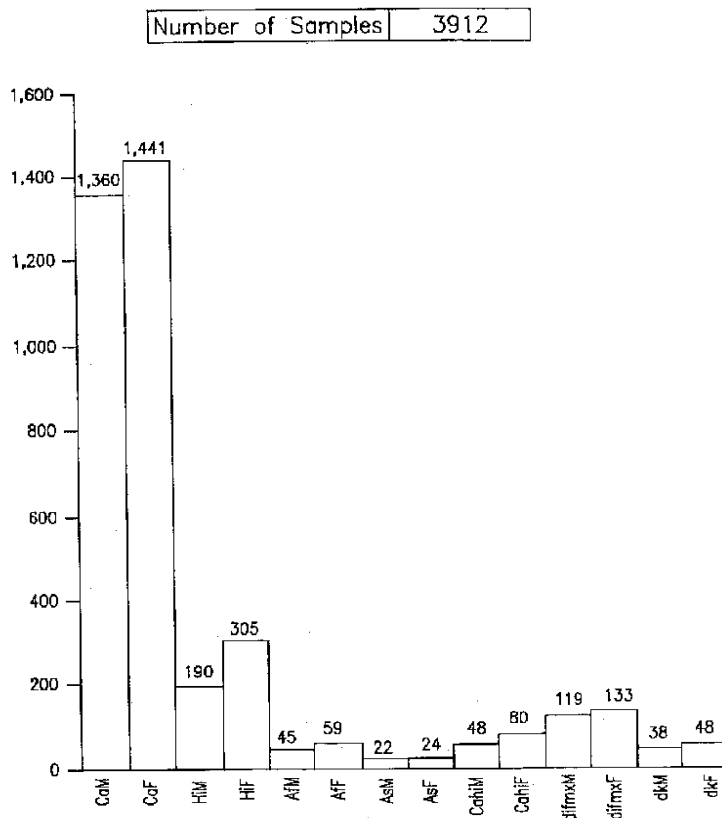
(60) Division of application No. 09/687,483, filed on Oct. 13, 2000.

Continuation-in-part of application No. 09/663,968, filed on Sep. 19, 2000.

(60) Provisional application No. 60/217,658, filed on Jul. 10, 2000. Provisional application No. 60/159,176, filed on Oct. 13, 1999. Provisional application No. 60/217,251, filed on Jul. 10, 2000.

Publication Classification(51) **Int. Cl.⁷ C12Q 1/68; G06F 19/00;
G01N 33/48; G01N 33/50**(52) **U.S. Cl. 435/6; 702/20**(57) **ABSTRACT**

Processes and methods for creating a database of genomic samples from healthy human donors, methods that use the database to identify and correlate polymorphic genetic markers and other markers with diseases and conditions are provided.

DNA Bank

DNA Bank

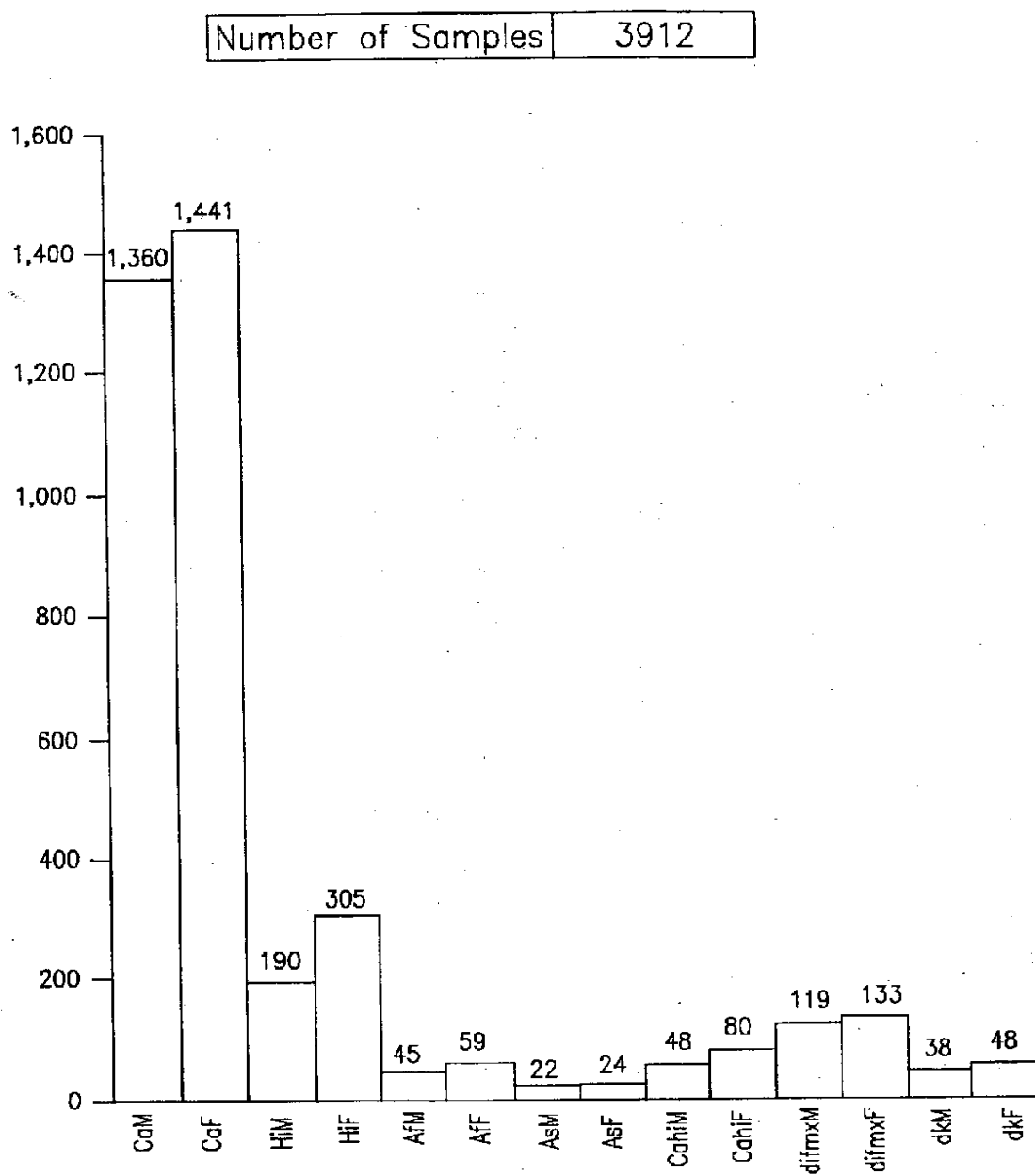


FIG. 1A

Caucasians

Number of Samples	2801
-------------------	------

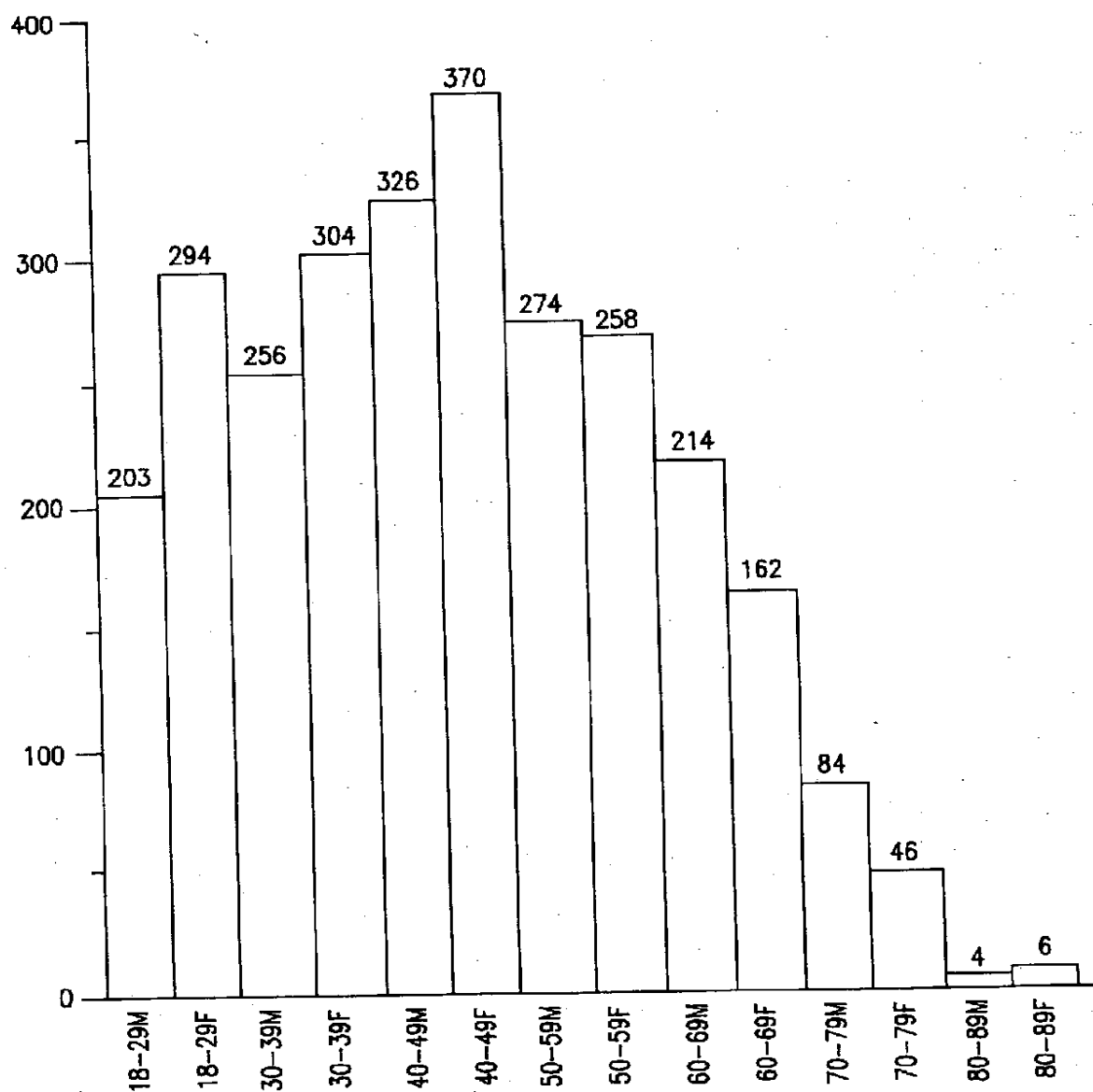


FIG. 1B

Hispanics

Number of Samples	495
-------------------	-----

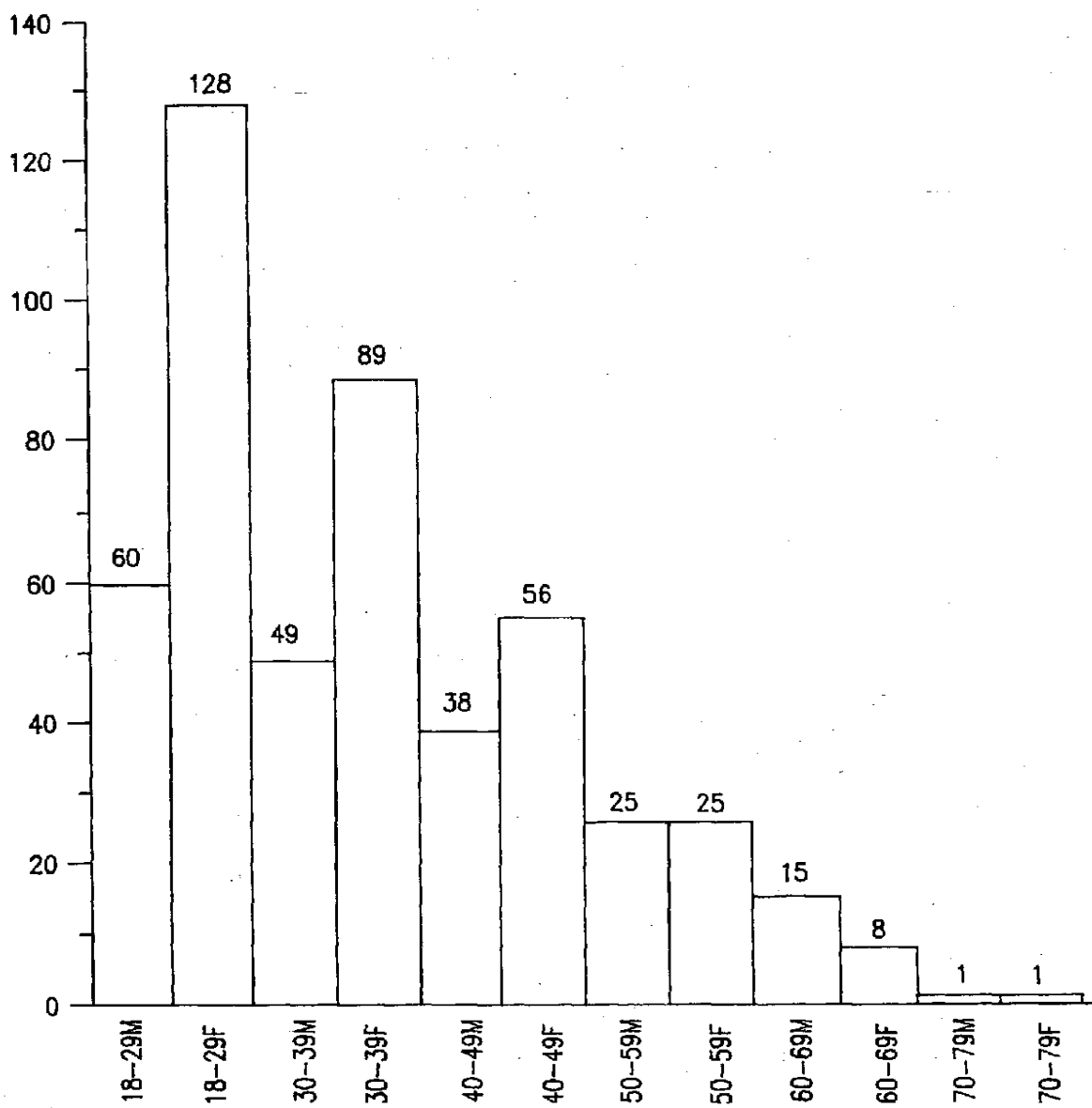
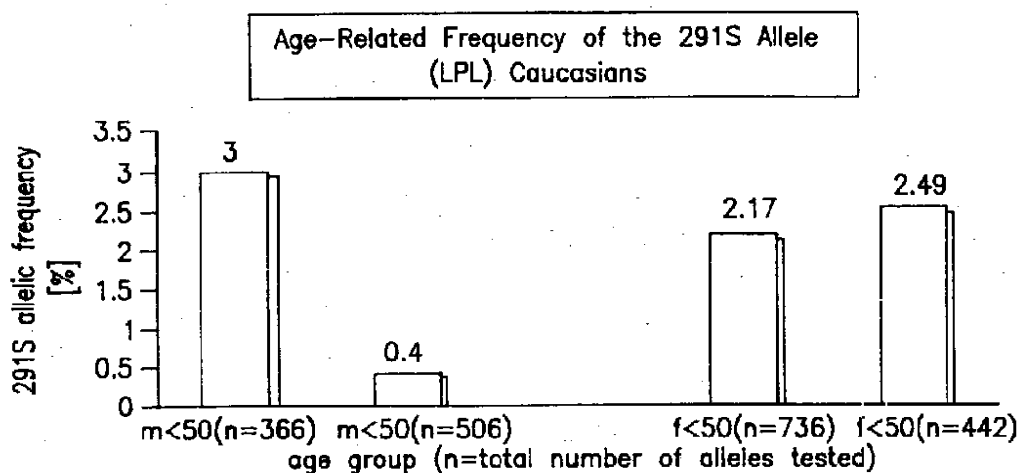
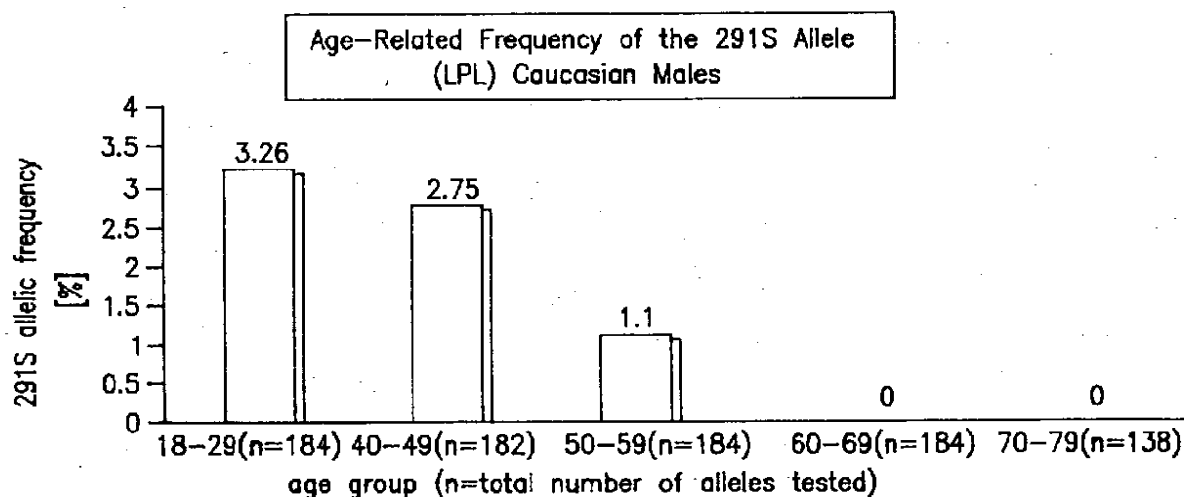


FIG. IC



age- and sex-distribution of the 291S allele of the lipoprotein lipase gene. A total of 436 males and 586 females were investigated.

FIG. 2A



Age- related distribution of the 291S allele of the lipoprotein lipase gene within the male Caucasian population. A total of 436 males were tested.

FIG. 2B

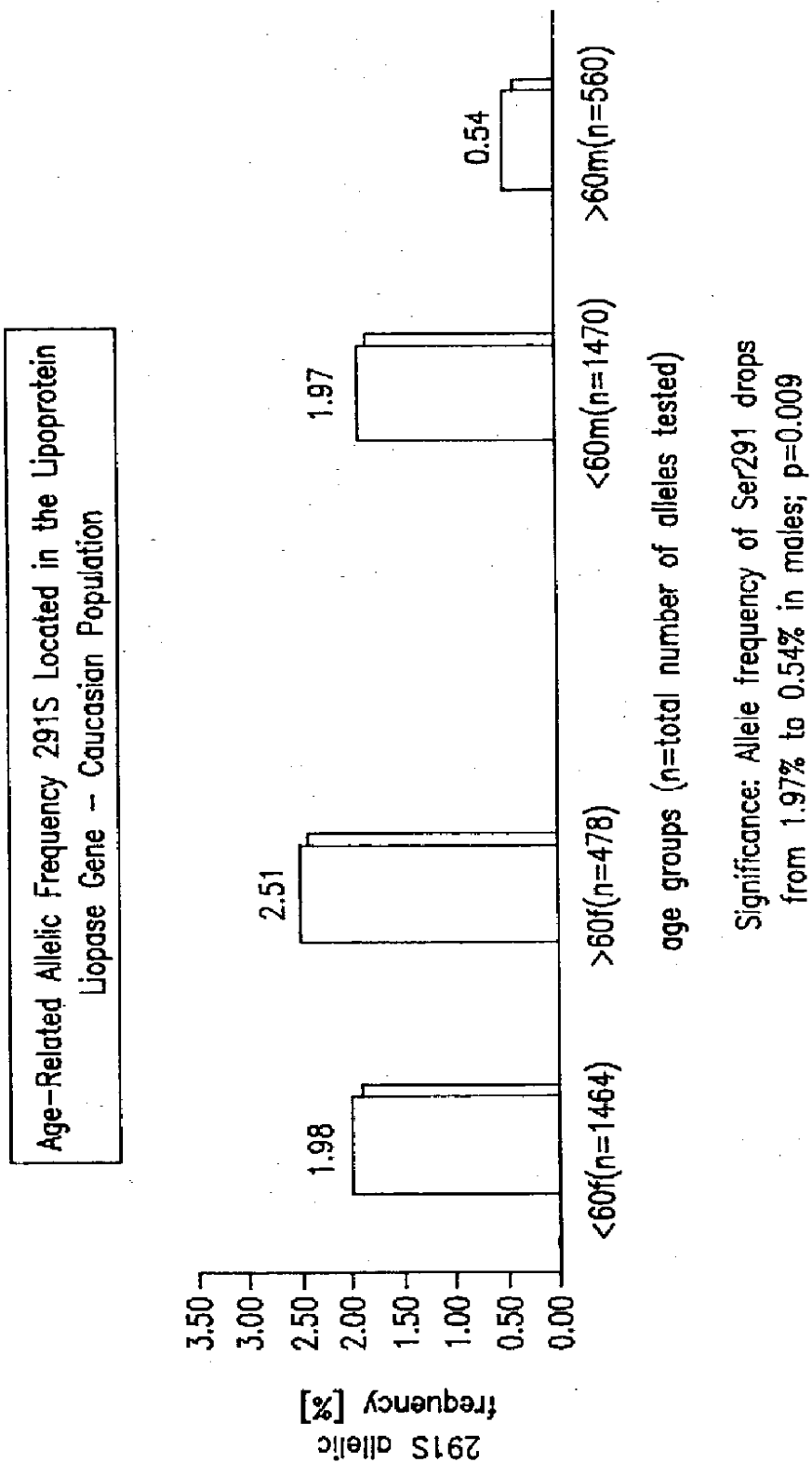


FIG. 2C

Questionnaire for
Population-Based
Sample Banking

Data Collection Form

Collection Information

Consent Form Signed Yes No

Date of Collection (MM/DD/YY) ___/___/98

Time of Sample Collection (nearest hour in 24 hour clock format) _____

Initials of Data Collector _____ Collecting Agency _____

(DO NOT COMPLETE: (For Date Entry Only) Sample _____ intact _____ lost _____ broken

Donor information

Sex: ☐ Male ☐ Female

Date of Birth (MM/YY) ___/___

In which state do you live? _____ How long have you lived there? _____ Years

What is your highest grade you completed in school?

☐ less than 8th grade ☐ 8th, 9th, 10th or 11th grade ☐ high school graduate or equivalency

☐ some college 2 yr. degree ☐ college graduate 4 yr degree ☐ post graduate education or degree

To the best of your knowledge what is the Ethnic Origin of your:

Father

Mother

- | | | |
|--------------------------|--------------------------|--|
| <input type="checkbox"/> | <input type="checkbox"/> | Caucasian (please check specific geographic area below if known) |
| <input type="checkbox"/> | <input type="checkbox"/> | Northern Europe (Austria, Denmark, Finland, France, Germany, Netherlands, Norway, Sweden, Switzerland, U.K.) |
| <input type="checkbox"/> | <input type="checkbox"/> | Southern Europe (Greece, Italy, Spain) |
| <input type="checkbox"/> | <input type="checkbox"/> | Eastern Europe (Czechoslovakia, Hungary, Poland, Russia, Yugoslavia) |
| <input type="checkbox"/> | <input type="checkbox"/> | Middle Eastern (Israel, Egypt, Iran, Iraq, Jordan, Syria, other Arab States) |
| <input type="checkbox"/> | <input type="checkbox"/> | African-American |
| <input type="checkbox"/> | <input type="checkbox"/> | Hispanic (please check specific geographic area below if known) |
| <input type="checkbox"/> | <input type="checkbox"/> | Mexico |
| <input type="checkbox"/> | <input type="checkbox"/> | Central America, South American |
| <input type="checkbox"/> | <input type="checkbox"/> | Cuba, Puerto Rico, other Caribbean |
| <input type="checkbox"/> | <input type="checkbox"/> | Asian (please check specific geographic area below if known) |
| <input type="checkbox"/> | <input type="checkbox"/> | Japanese |
| <input type="checkbox"/> | <input type="checkbox"/> | Chinese |
| <input type="checkbox"/> | <input type="checkbox"/> | Korean |
| <input type="checkbox"/> | <input type="checkbox"/> | Vietnamese |
| <input type="checkbox"/> | <input type="checkbox"/> | other Asian |
| <input type="checkbox"/> | <input type="checkbox"/> | Other _____ |
| <input type="checkbox"/> | <input type="checkbox"/> | Don't know |

Health information: Have you or has anyone in your immediate family (parents, brothers, sisters, or your children) had the following? Check all that apply

Disease: _____ You _____ Mother _____ Father _____ Sister _____ Brother _____ Child _____

Heart Disease Stroke or Arteriosclerosis
Cancer (Specify type if known)
Alzheimer's Disease or Dementia
Chronic inflammatory or Autoimmune Disease
Nervous System Disease like Multiple Sclerosis
Other (please specify)

Additional health information details you would like to provide:

FIG. 3

Sample Banks

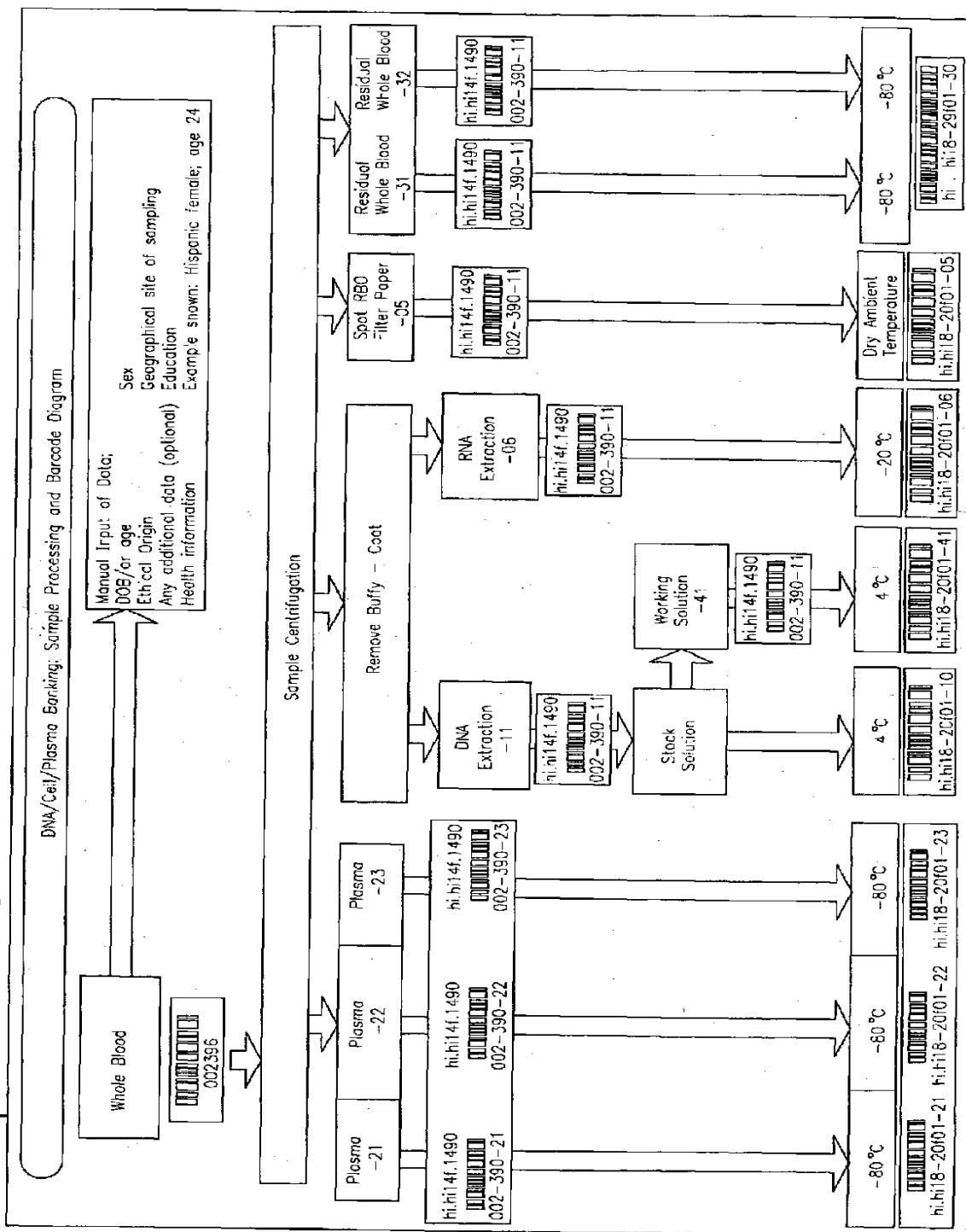


FIG. 4

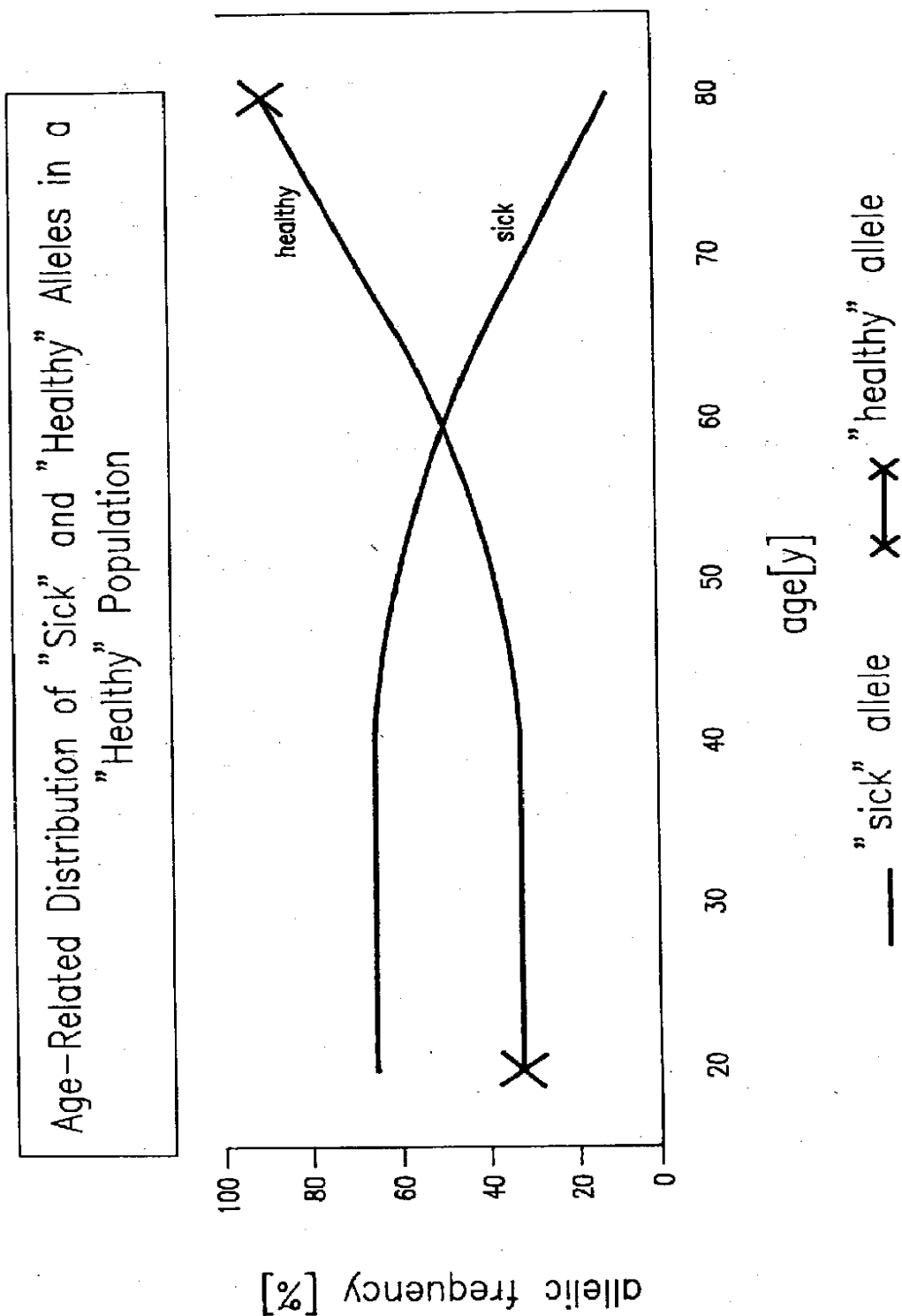


FIG. 5

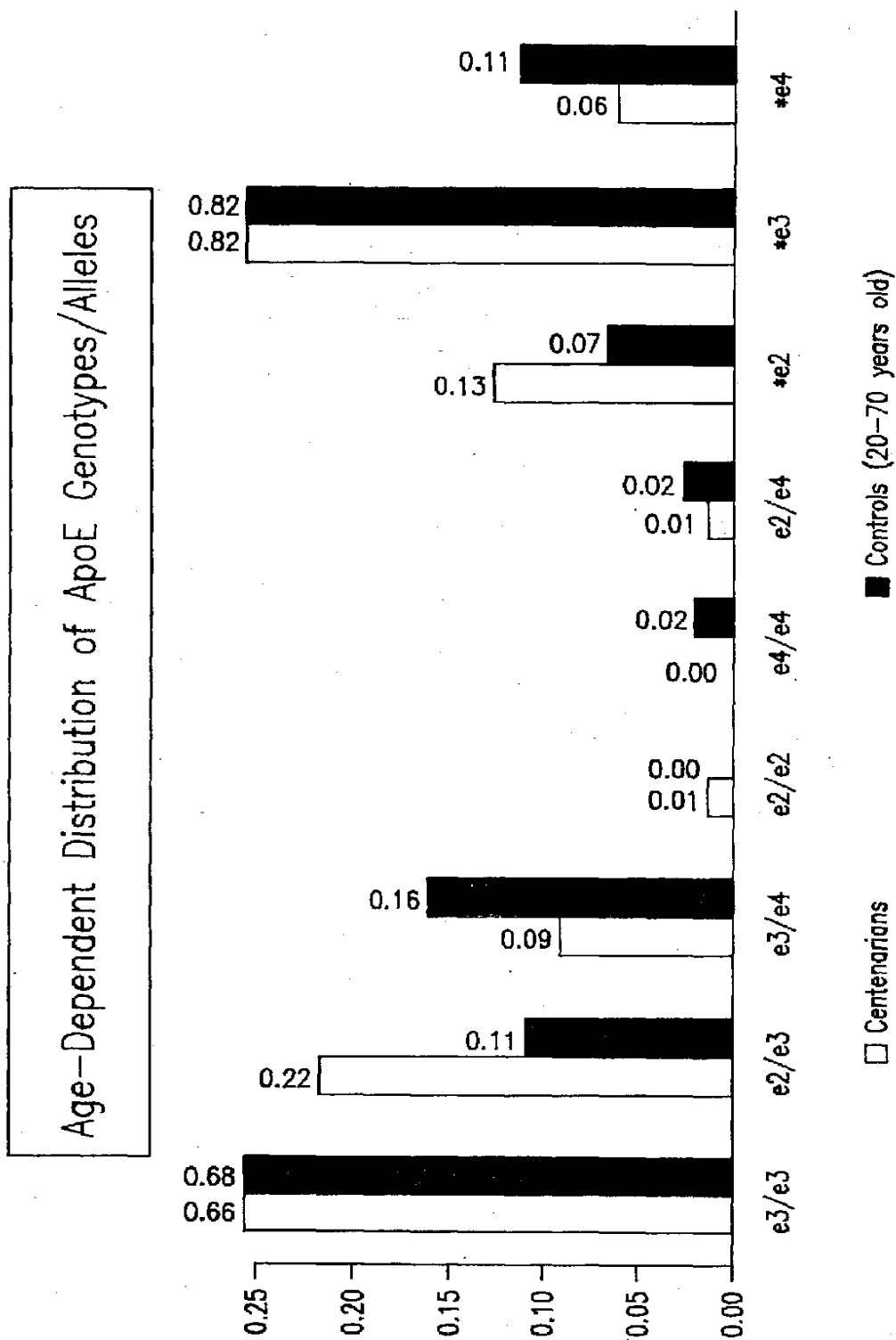


FIG. 6

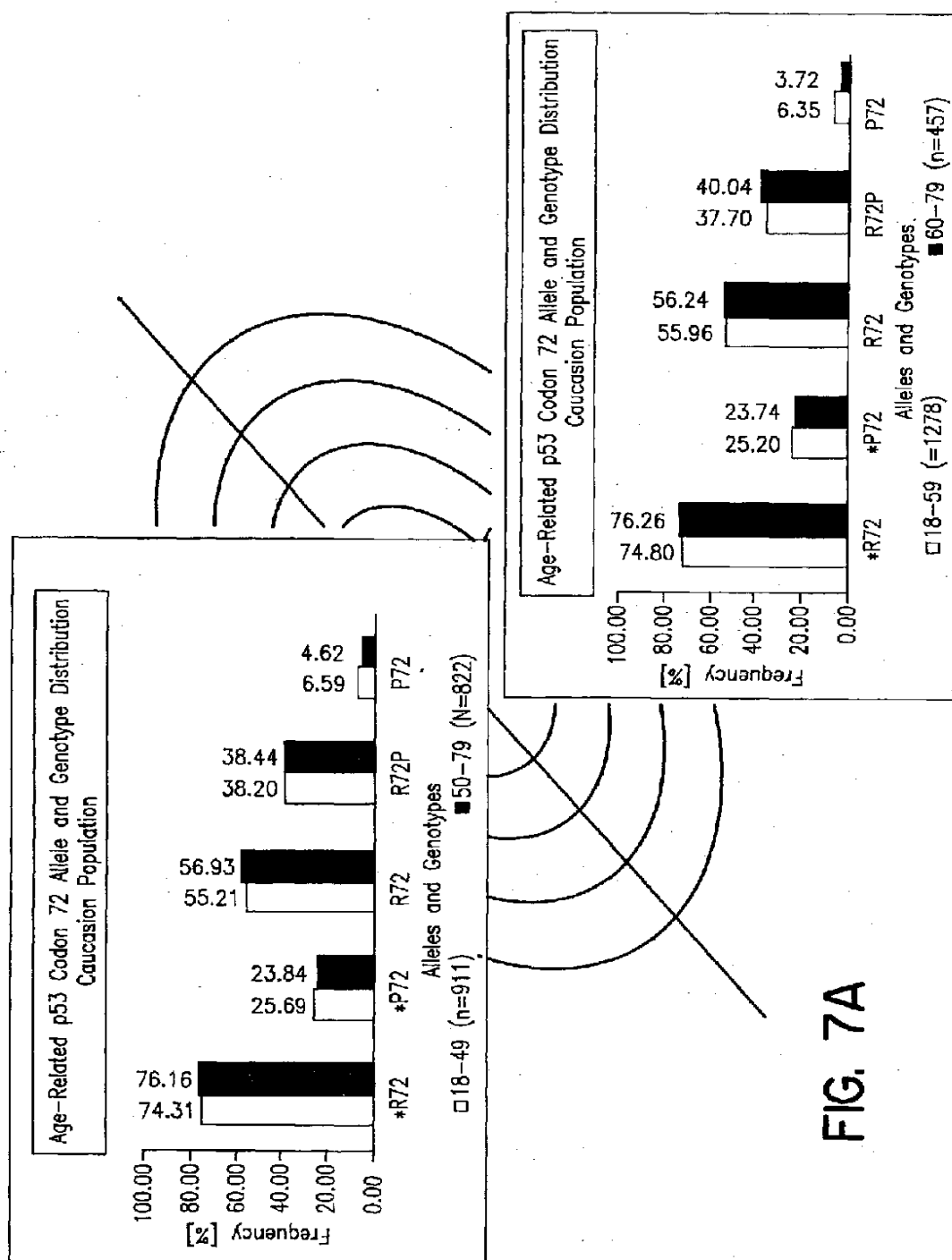


FIG. 7A

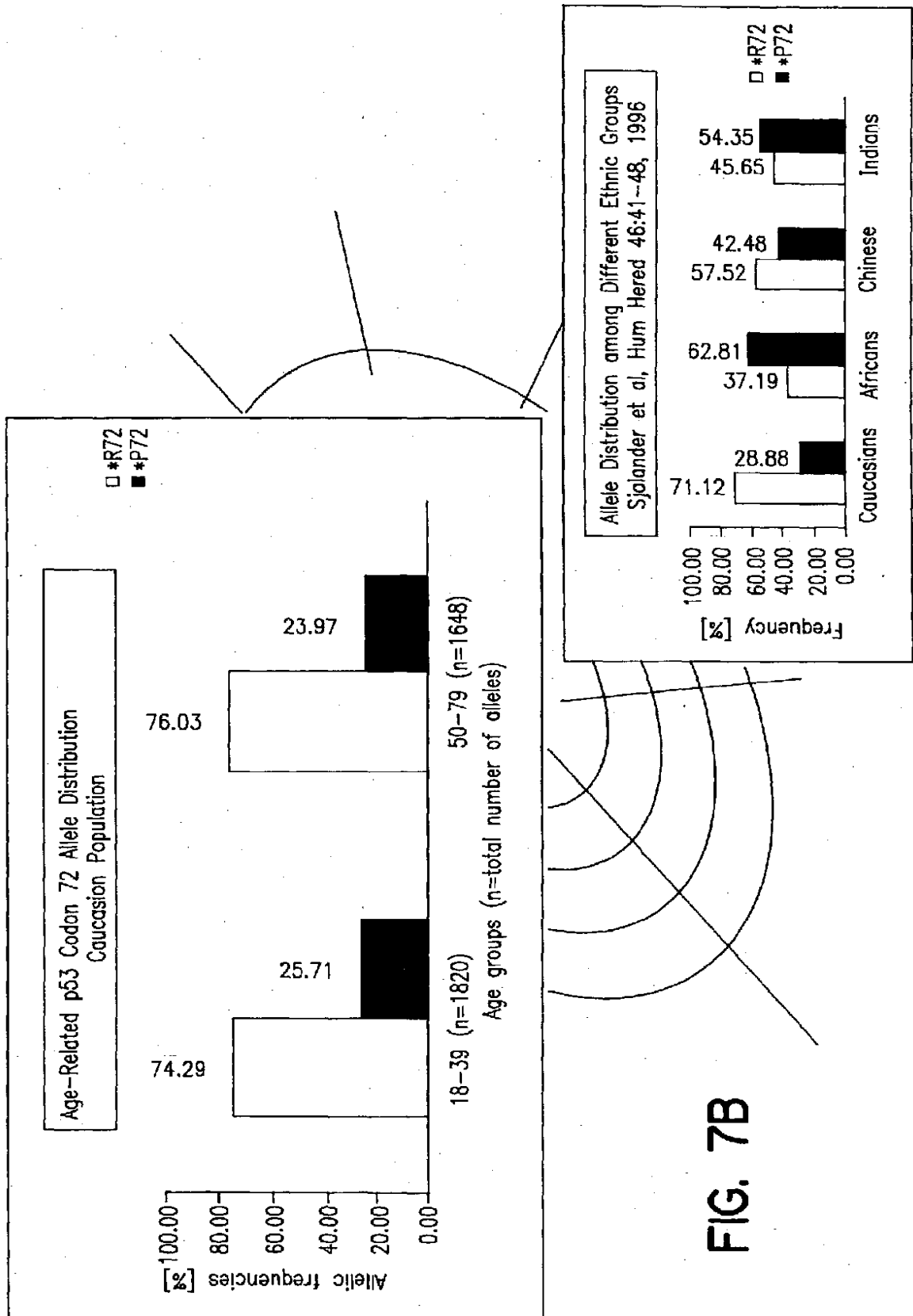
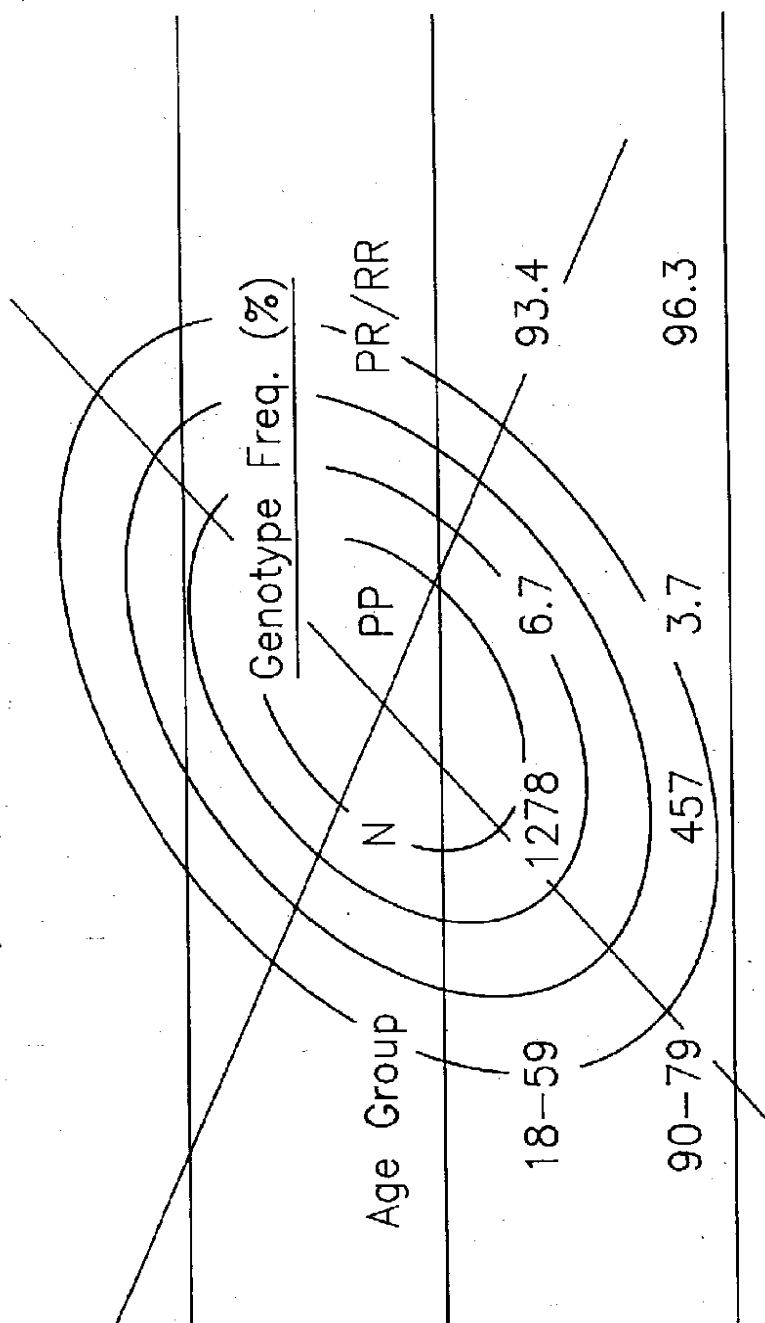


FIG. 7B

FIG. 7C

P53 PP vs. PR/RR Genotype Distribution
By Age cut point = 59



Sample Size : 1735
 χ^2 : 5.2 (1 d.f.), P = 0.02

Genomic Organization of the p53 Gene

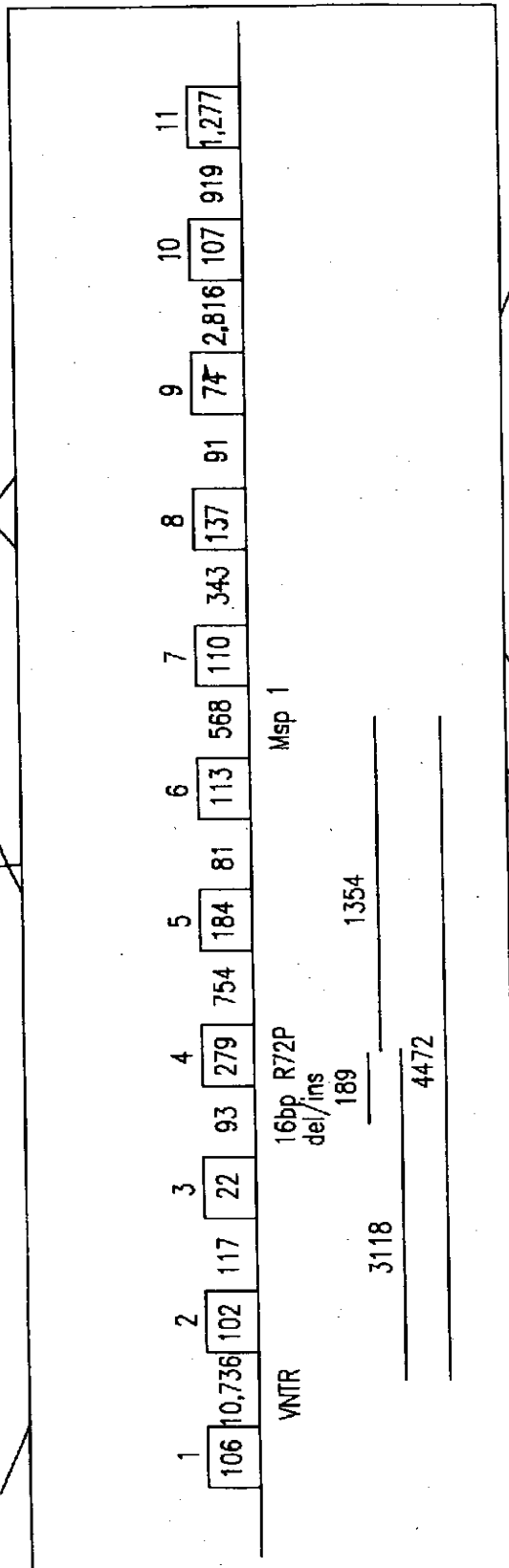


FIG. 7D

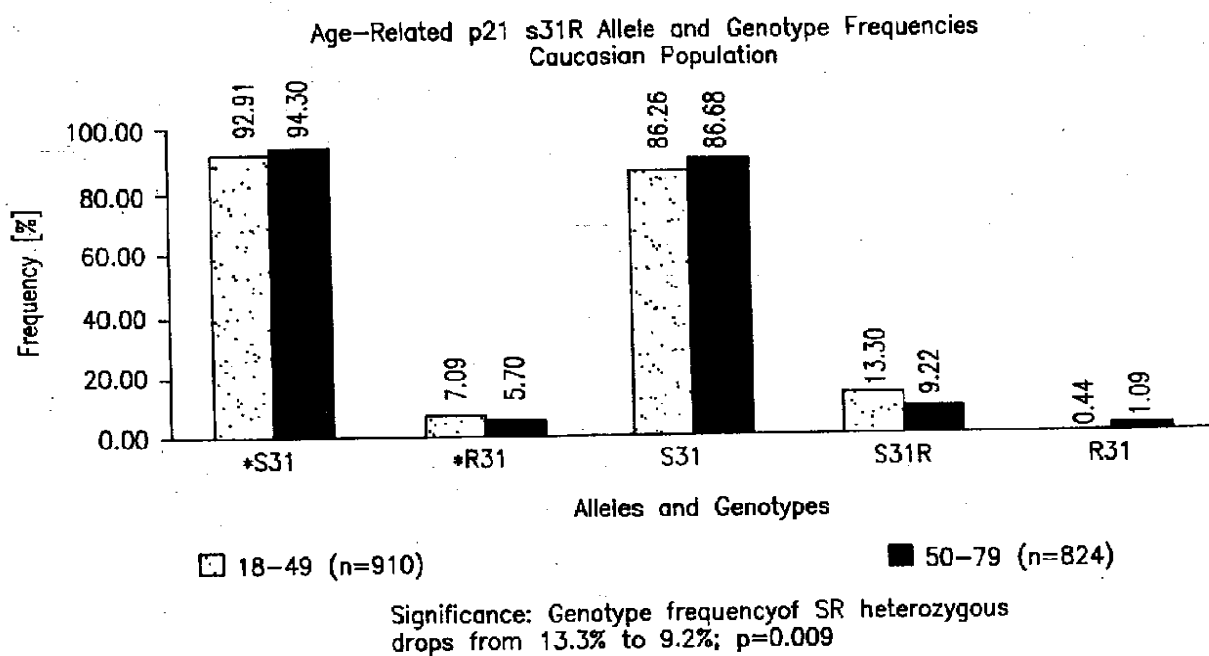


FIG. 8

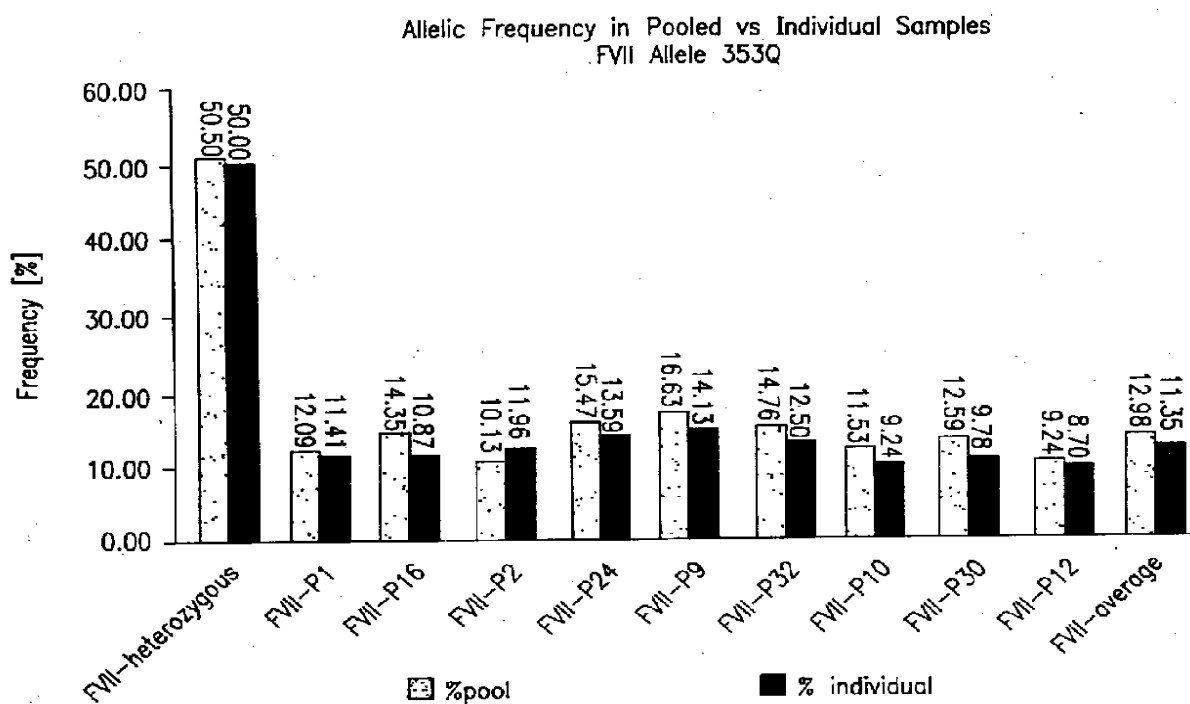


FIG. 9

Allelic Frequency in Pooled vs Individual Samples
CETP Allele 405V

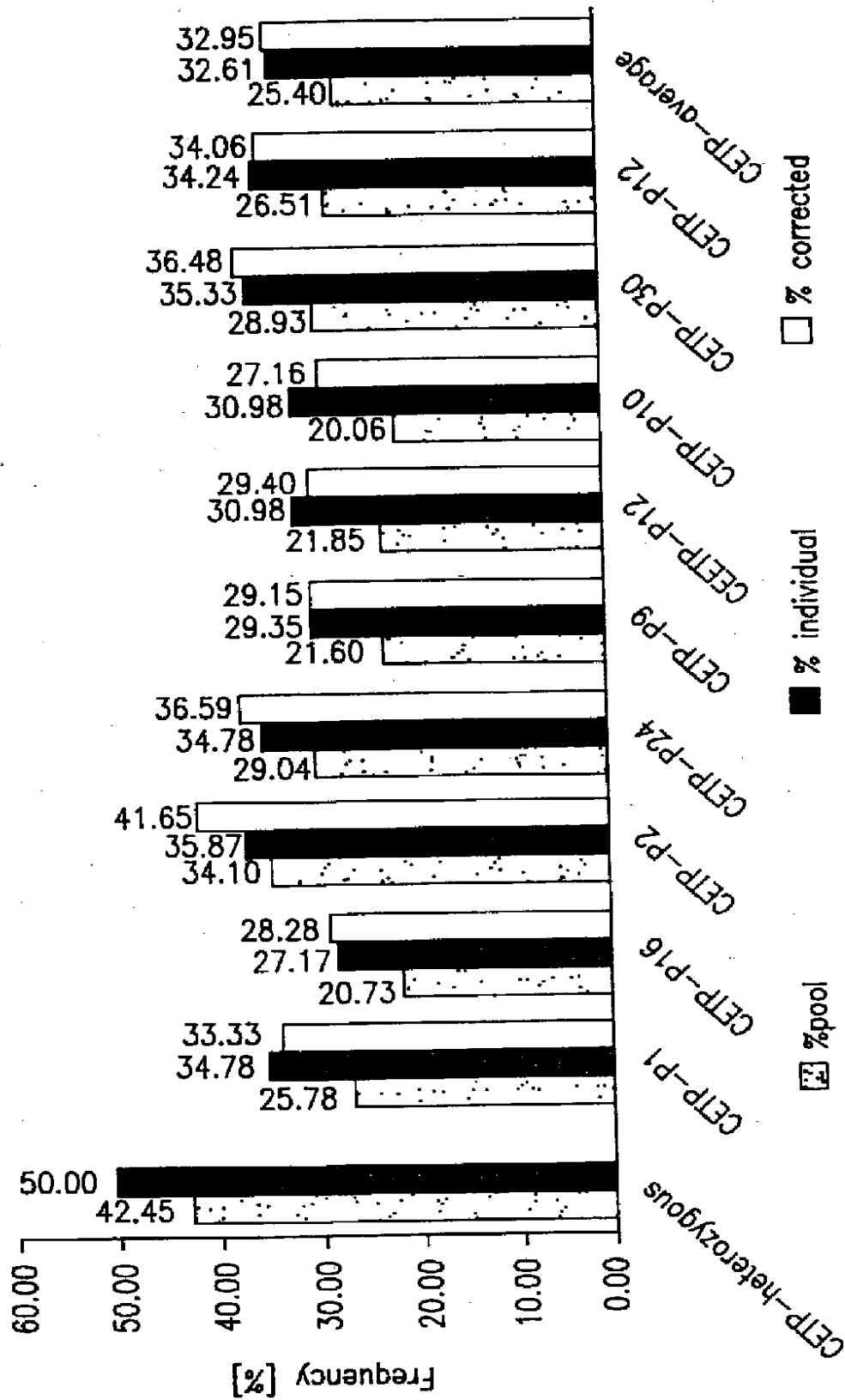


FIG. 10

Allelic Frequency in Pooled vs Individual Samples
Allele PAI-1 5G

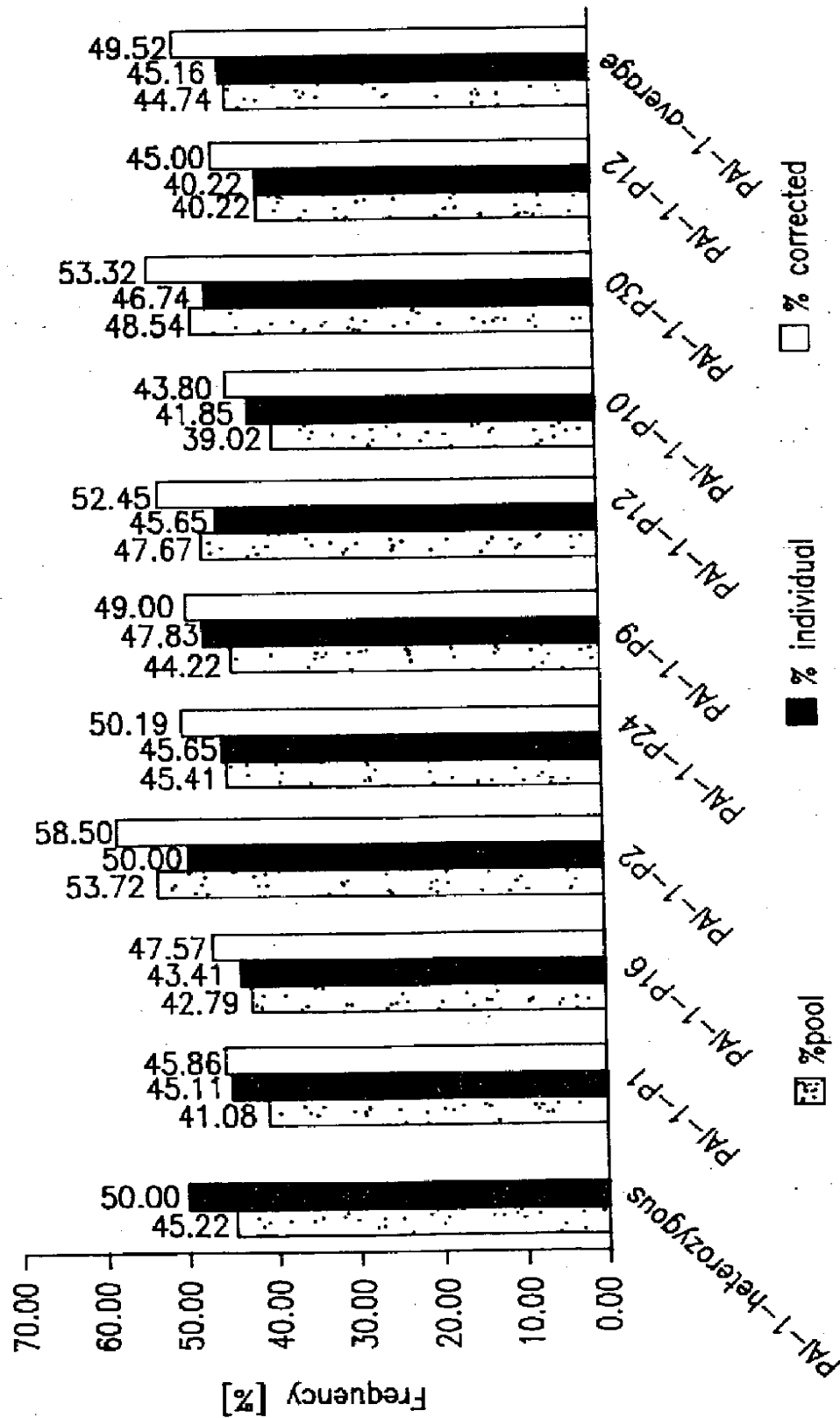


FIG. 11

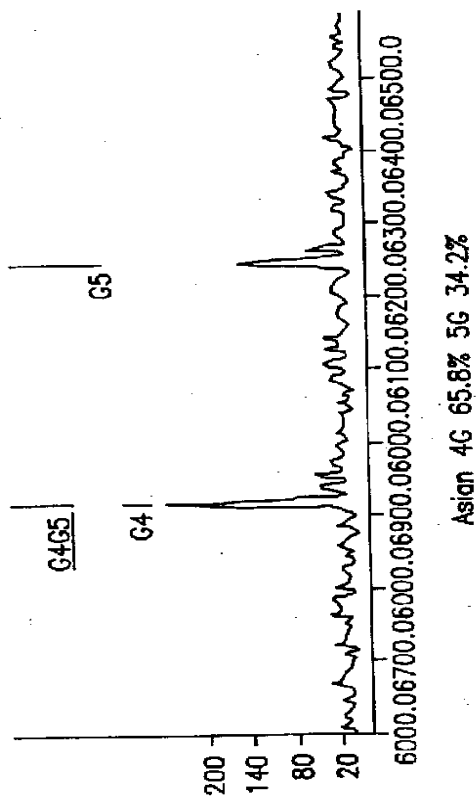


FIG. 12A

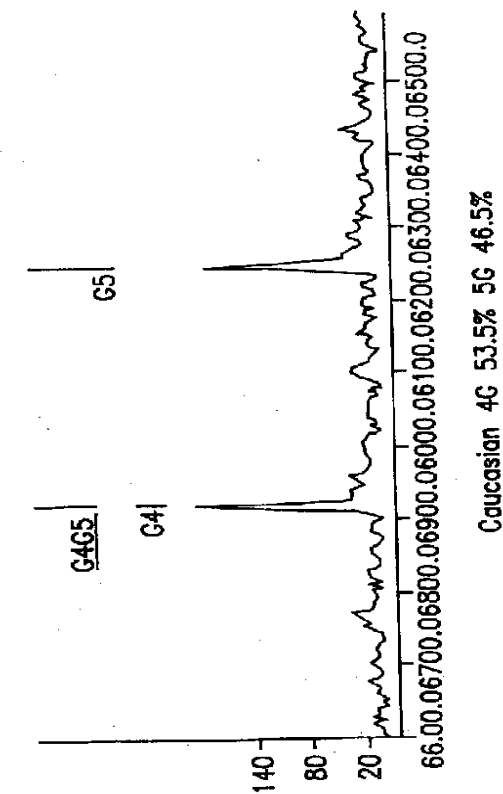


FIG. 12C

FIG. 12B

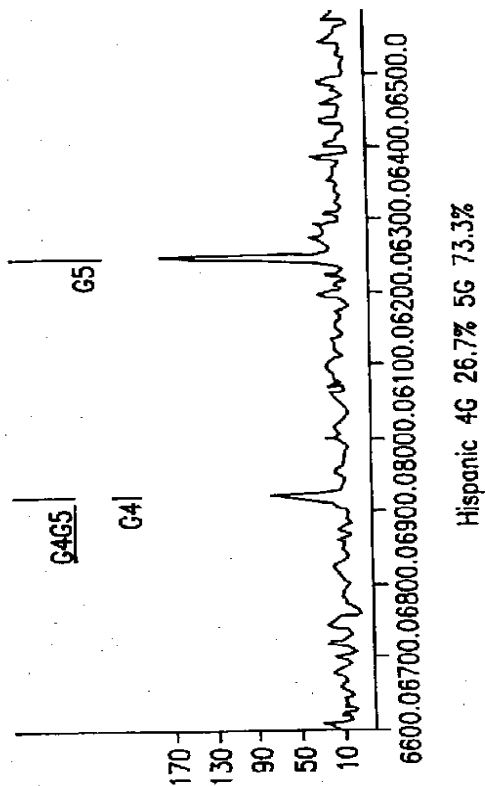
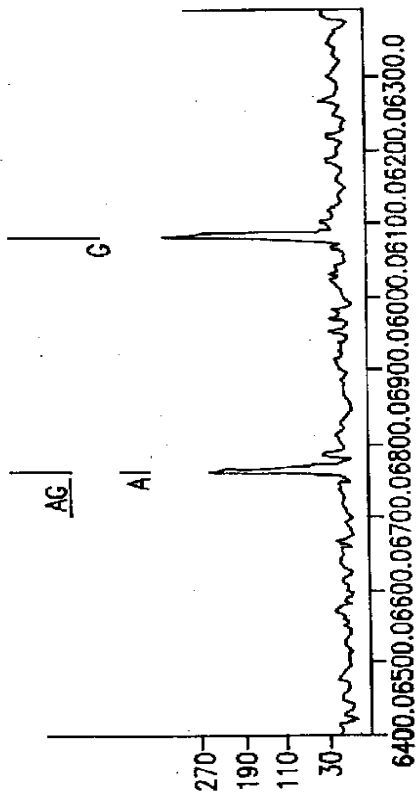
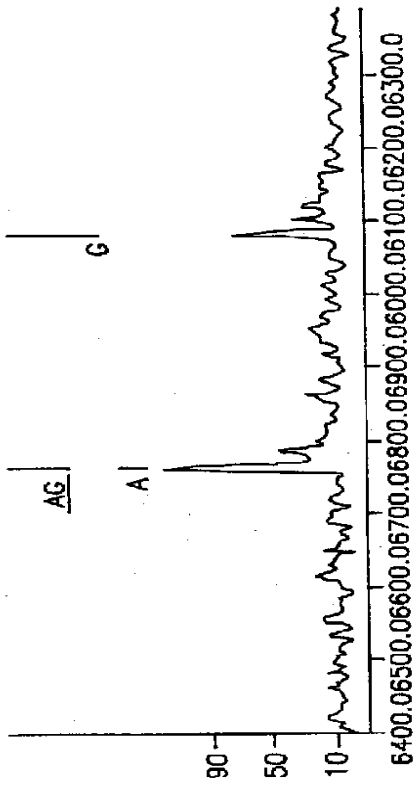


FIG. 12D



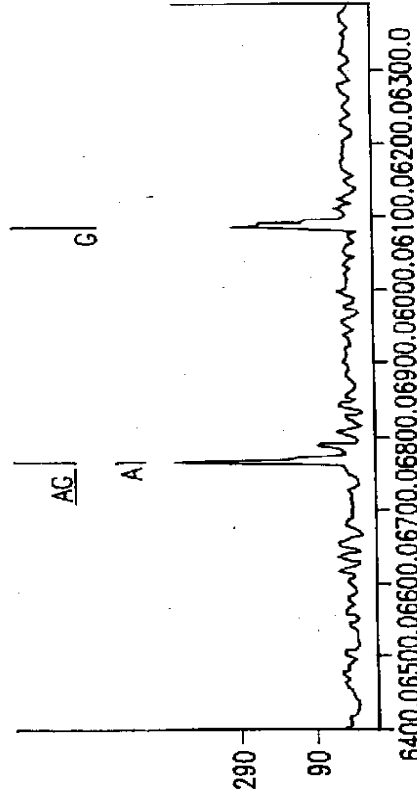
African Ile 43.6% Val 56.4%

FIG. 13A



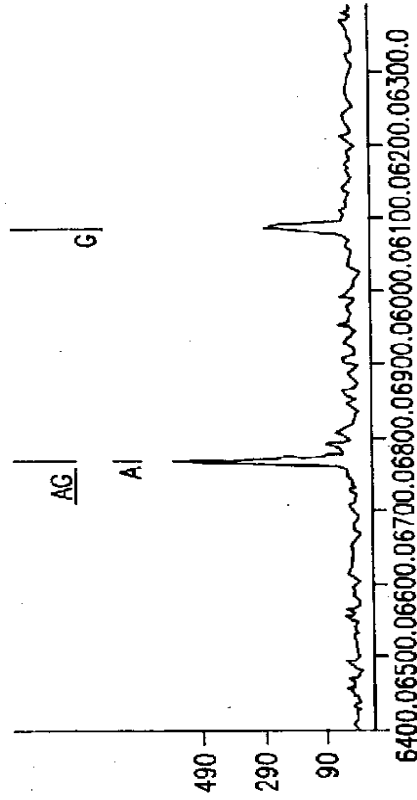
Asian Ile 60.7% Val 39.3%

FIG. 13B



Caucasian Ile 63.0% Val 37.0%

FIG. 13C



Hispanic Ile 70.1% Val 29.9%

FIG. 13D

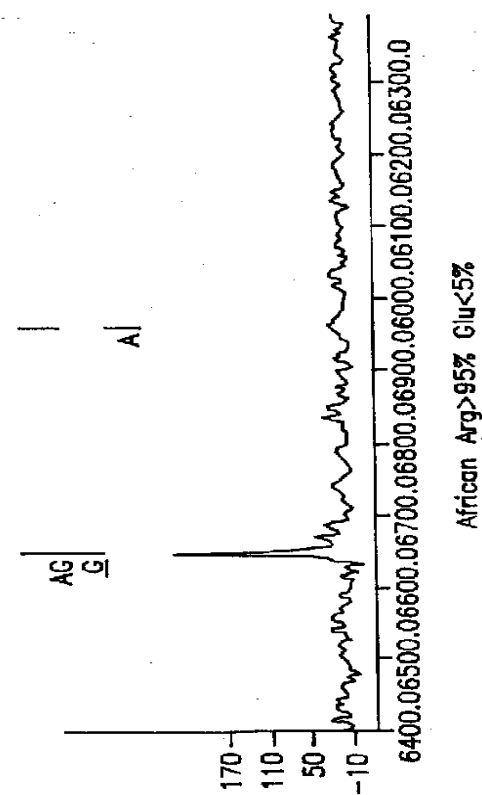


FIG. 14A

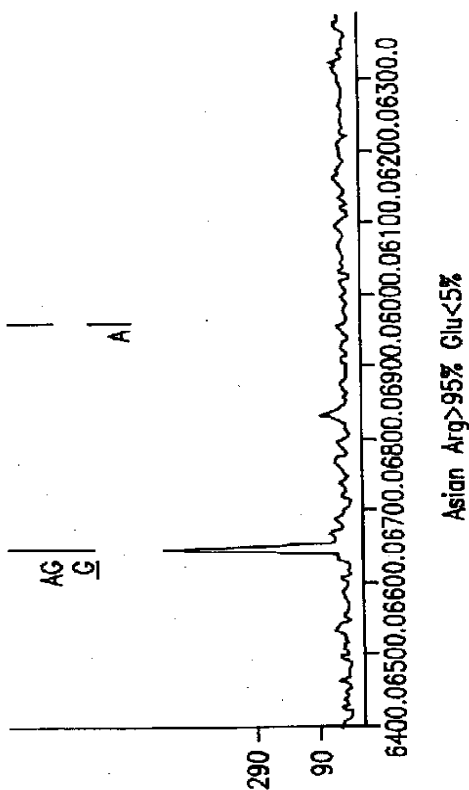


FIG. 14B

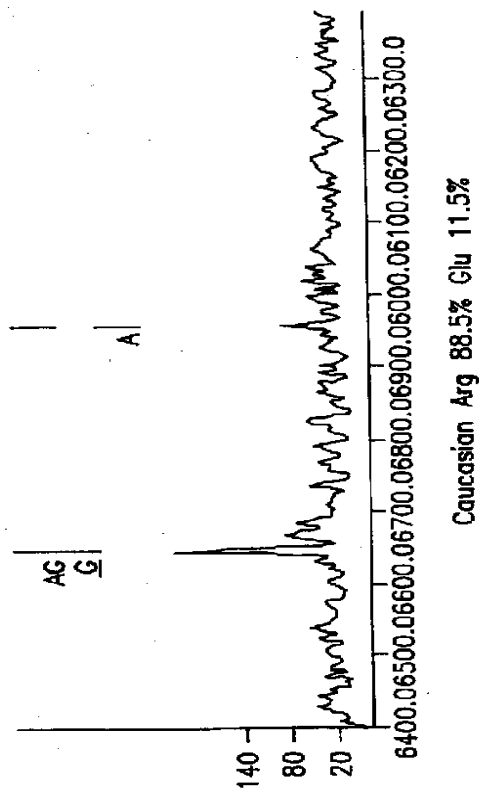


FIG. 14C

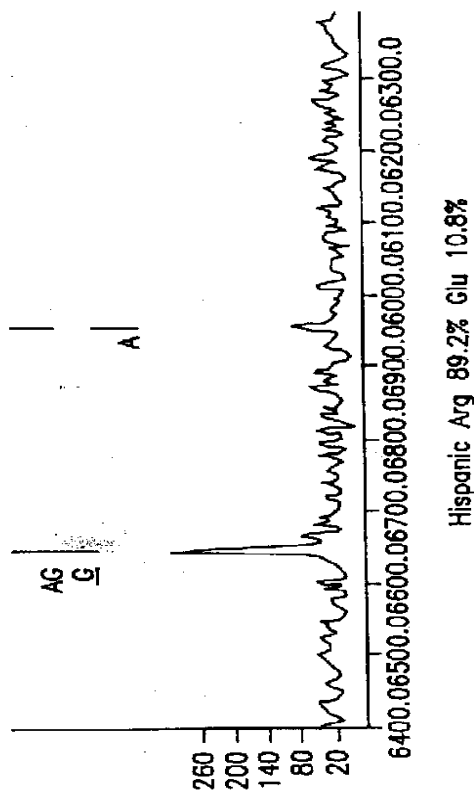


FIG. 14D

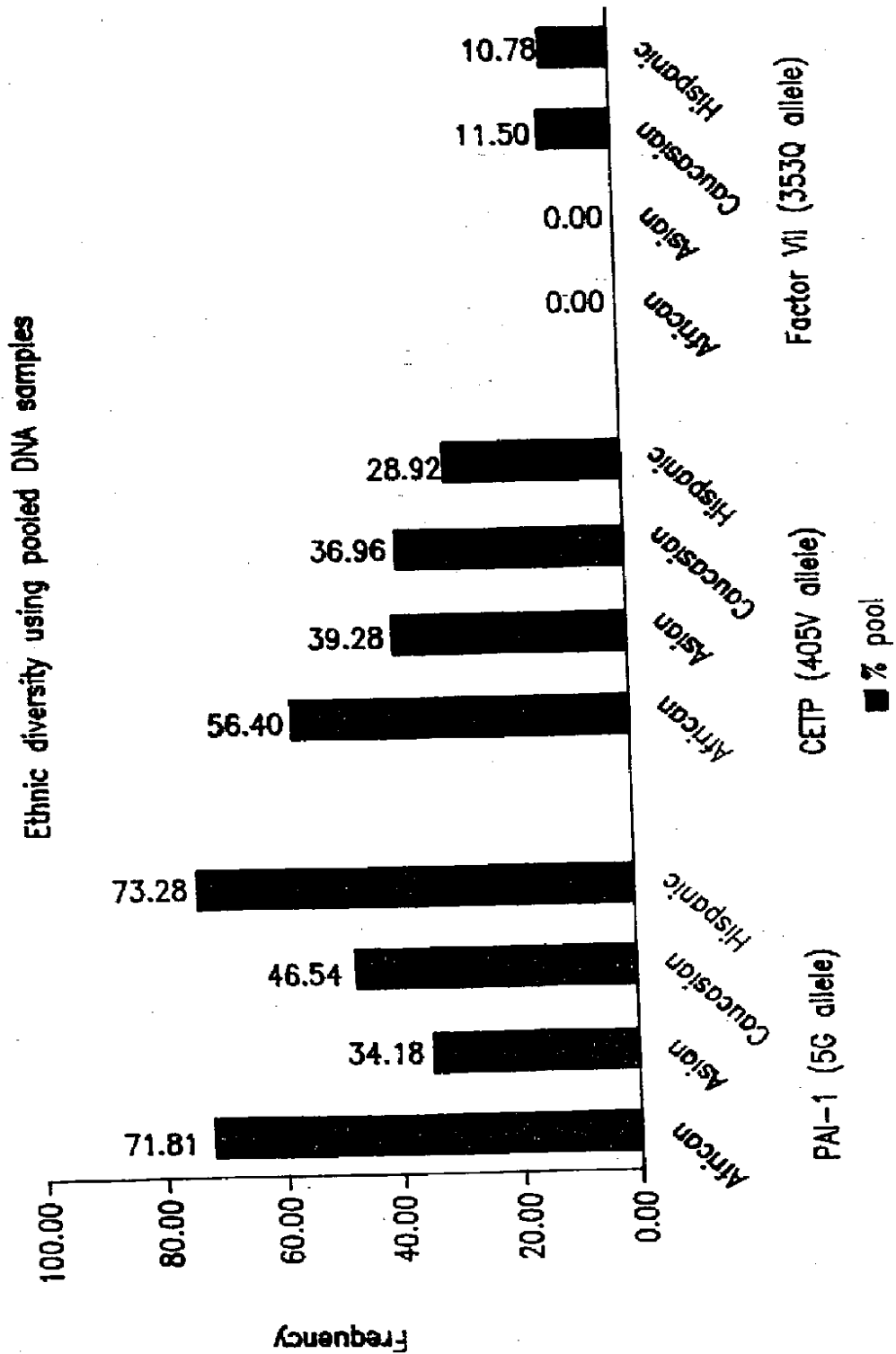


FIG. 15

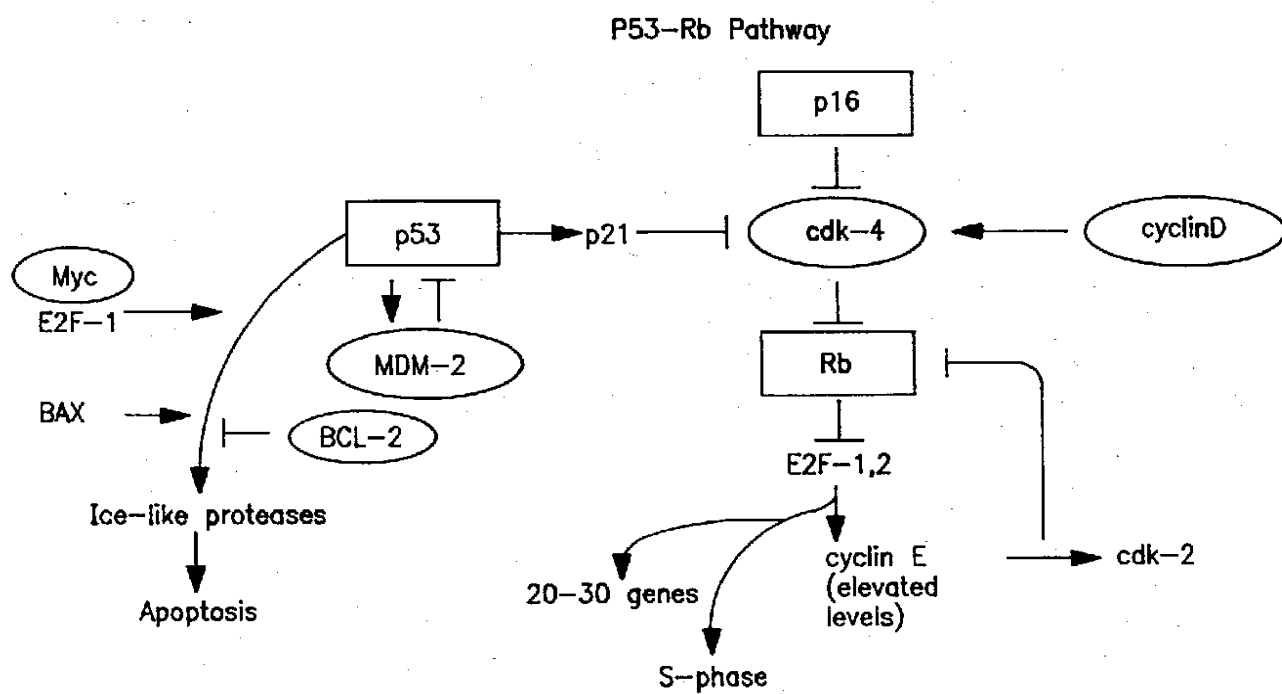


FIG. 16

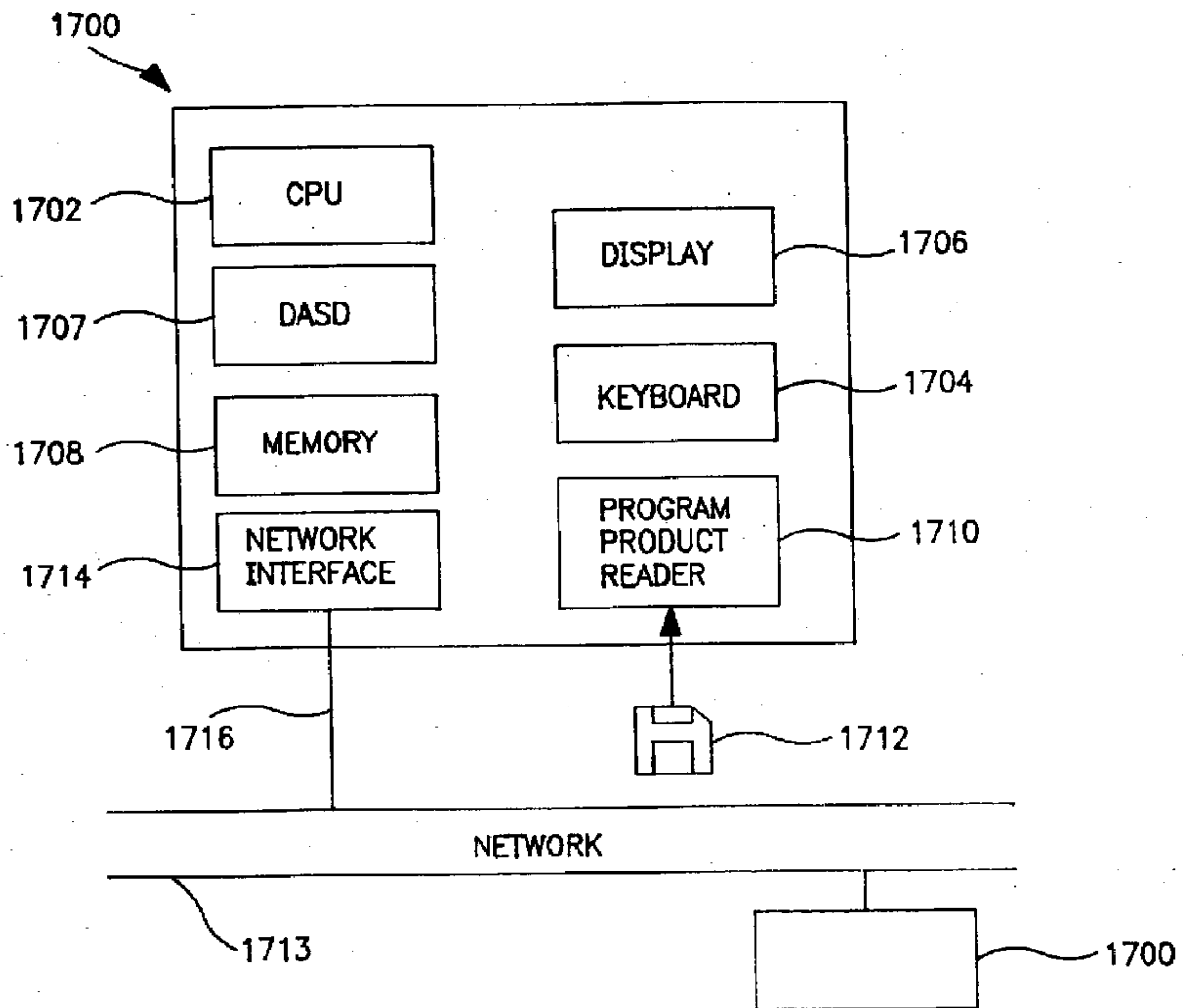


FIG. 17

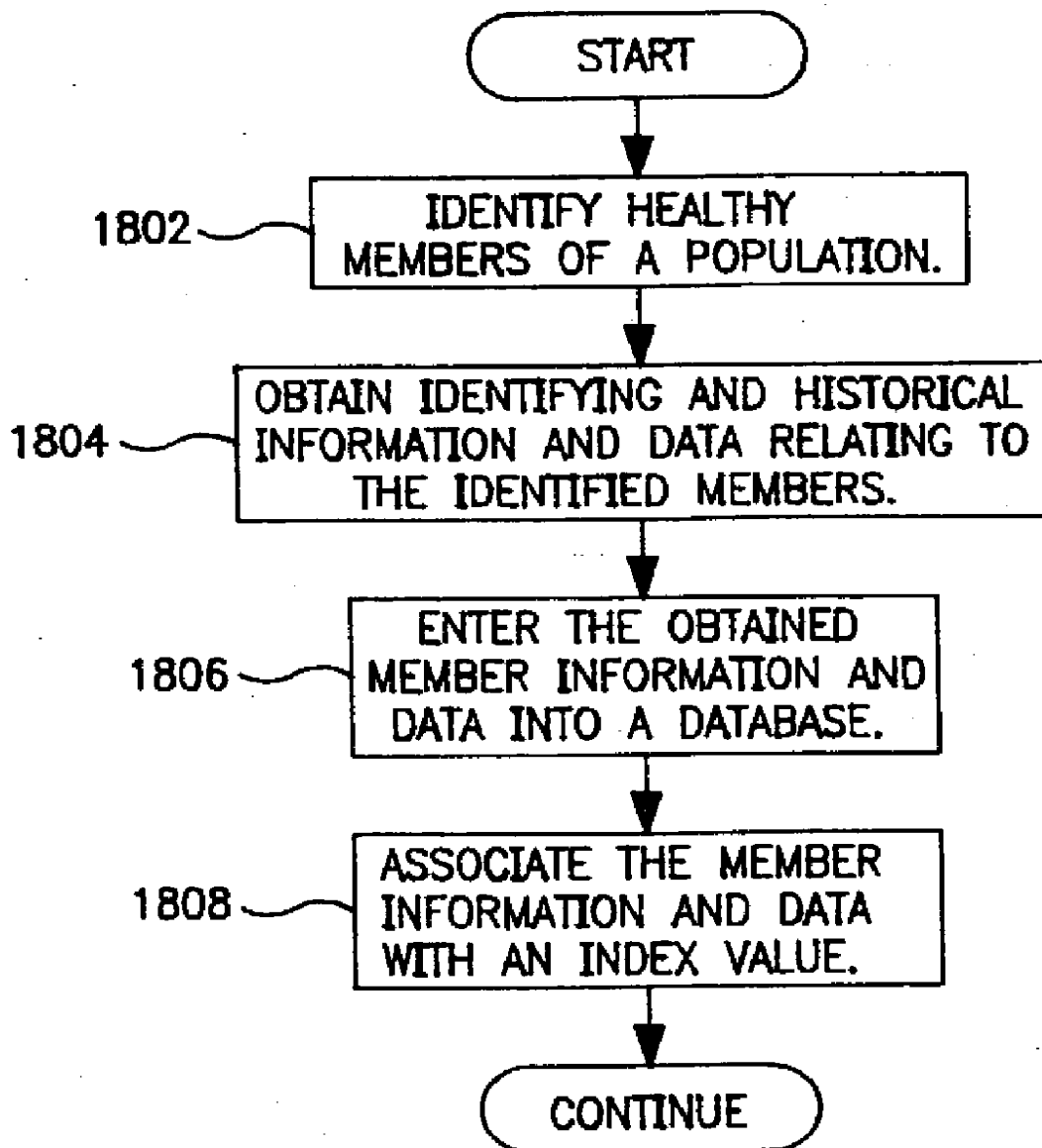


FIG. 18

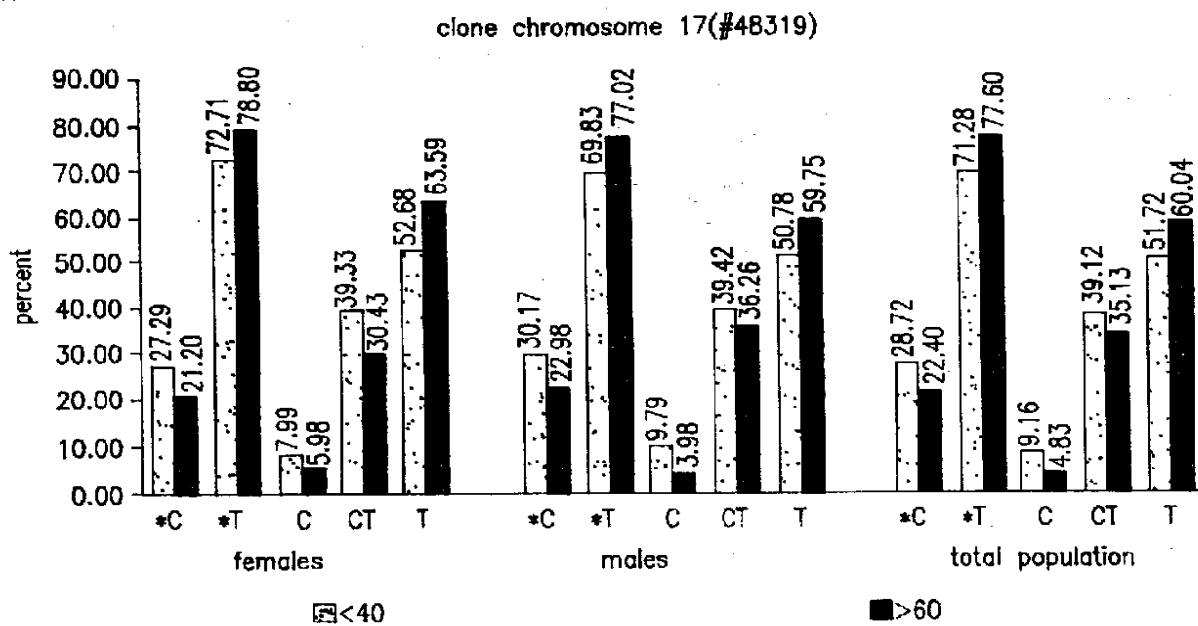


FIG. 19

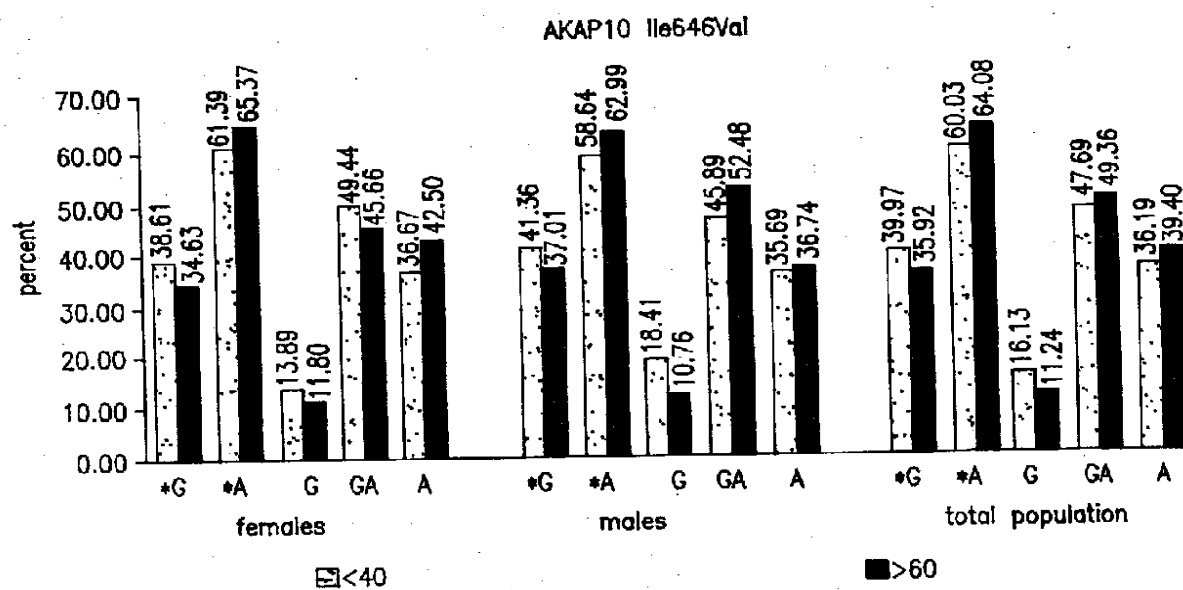


FIG. 20

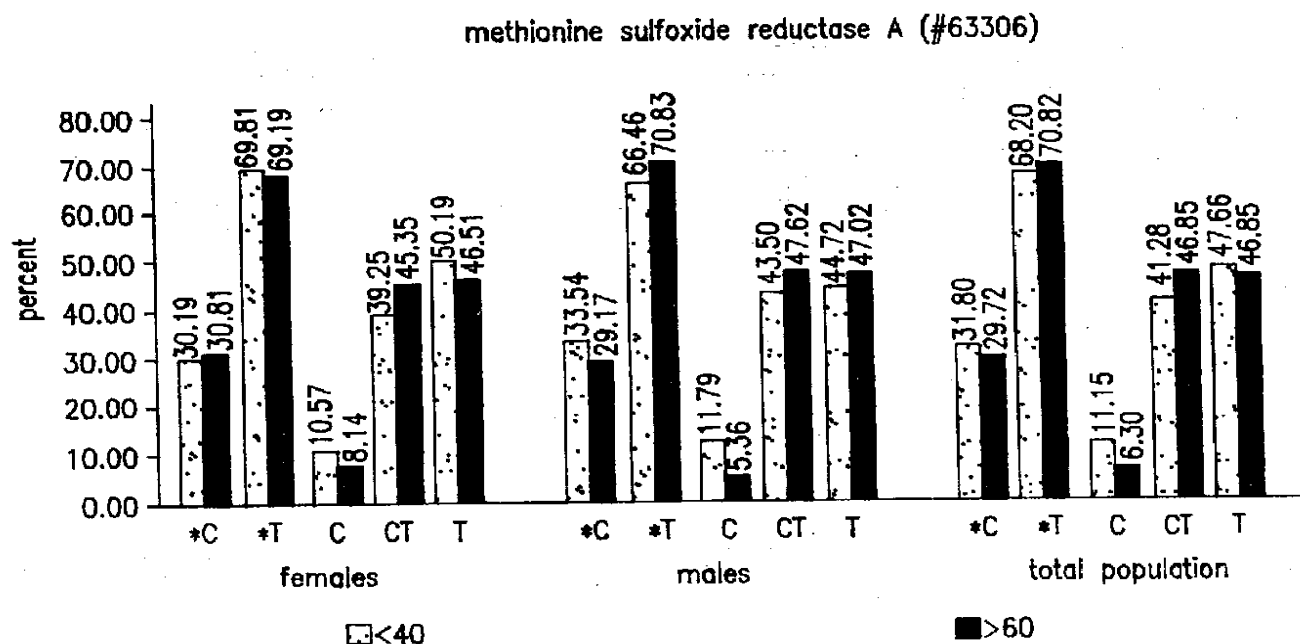


FIG. 21

Collection Information									
Consent Form Signed <input type="checkbox"/> Yes <input type="checkbox"/> No									
Date of Collection			Time of Sample Collection (nearest hour, in 24 hour clock format)		Initials		Initials of Data Collector		
Month	Day	Year					(DO NOT COMPLETE; for data entry only)		
JAN							Sample; <input type="checkbox"/> Intact <input type="checkbox"/> Lost <input type="checkbox"/> Broken		
FEB							Volume (ml) <input type="checkbox"/>		
MAR							<input type="checkbox"/>		
APR							<input type="checkbox"/>		
MAY							<input type="checkbox"/>		
JUN							<input type="checkbox"/>		
JUL							<input type="checkbox"/>		
AUG							<input type="checkbox"/>		
SEP							<input type="checkbox"/>		
OCT							<input type="checkbox"/>		
NOV							<input type="checkbox"/>		
DEC							<input type="checkbox"/>		

Donor Information														
Date of Birth			Height Ft. Inches		Weight (lb)		What Physical activity do you do on a regular basis?		Are you a vegetarian?		If Female: How many times have you been pregnant?		How many times did you give birth?	
Month	Day	Year					<input type="checkbox"/> Running <input type="checkbox"/> Swimming <input type="checkbox"/> Biking <input type="checkbox"/> Gymnastics <input type="checkbox"/> Other <input type="checkbox"/> None		<input type="checkbox"/> Yes <input type="checkbox"/> No		<input type="checkbox"/>		<input type="checkbox"/>	
JAN														
FEB														
MAR														
APR														
MAY														
JUN														
JUL														
AUG														
SEP														
OCT														
NOV														
DEC														

To the best of your knowledge, what is the Ethnic Origin of your:

Father	Mother
<input type="checkbox"/>	<input type="checkbox"/> Caucasian (please mark specific geographic area below if known)
<input type="checkbox"/>	<input type="checkbox"/> Northern Europe (Austria, Denmark, Finland, France, Germany, Netherlands, Norway, Sweden, Switzerland, UK)
<input type="checkbox"/>	<input type="checkbox"/> Southern Europe (Greece, Italy, Spain, Turkey)
<input type="checkbox"/>	<input type="checkbox"/> Eastern Europe (Czechoslovakia, Hungary, Poland, Russia, Yugoslavia)
<input type="checkbox"/>	<input type="checkbox"/> Middle Eastern (Israel, Egypt, Iran, Iraq, Jordan, Syria, Other Arab States)
<input type="checkbox"/>	<input type="checkbox"/> African-American
<input type="checkbox"/>	<input type="checkbox"/> Hispanic (please mark specific geographic area below if known)
<input type="checkbox"/>	<input type="checkbox"/> Mexico
<input type="checkbox"/>	<input type="checkbox"/> Central America, South America
<input type="checkbox"/>	<input type="checkbox"/> Cuba, Puerto Rico, other Caribbean
<input type="checkbox"/>	<input type="checkbox"/> Asian (please mark specific geographic area below if known)
<input type="checkbox"/>	<input type="checkbox"/> Japanese
<input type="checkbox"/>	<input type="checkbox"/> Chinese
<input type="checkbox"/>	<input type="checkbox"/> Korean
<input type="checkbox"/>	<input type="checkbox"/> Vietnamese
<input type="checkbox"/>	<input type="checkbox"/> Filipino
<input type="checkbox"/>	<input type="checkbox"/> Native American
<input type="checkbox"/>	<input type="checkbox"/> Other _____
<input type="checkbox"/>	<input type="checkbox"/> Don't know

How long have you lived there?	Years	What is your highest grade you completed in school?	Mother Deceased? Cause of Death Mother:	Father Deceased? Cause of Death Father:
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/> less than 8th grade	<input type="checkbox"/> Yes	<input type="checkbox"/> Yes
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/> 8th, 9th, 10th, or 11th grade	<input type="checkbox"/> No	<input type="checkbox"/> No
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/> high school graduate or equivalency	If Yes at what age?	If Yes at what age?
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/> some college, 2yr degree	< 29	< 29
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/> college graduate, 4yr degree	30-39	30-39
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/> post graduate education or degree	40-49	40-49
<input type="checkbox"/>	<input type="checkbox"/>		50-59	50-59
<input type="checkbox"/>	<input type="checkbox"/>		60-69	60-69
<input type="checkbox"/>	<input type="checkbox"/>		70-79	70-79
<input type="checkbox"/>	<input type="checkbox"/>		80-89	80-89
<input type="checkbox"/>	<input type="checkbox"/>		≥ 90	≥ 90
<input type="checkbox"/>	<input type="checkbox"/>		Heart Disease	Heart Disease
<input type="checkbox"/>	<input type="checkbox"/>		Cancer	Cancer
<input type="checkbox"/>	<input type="checkbox"/>		Stroke	Stroke
<input type="checkbox"/>	<input type="checkbox"/>		Accident	Accident
<input type="checkbox"/>	<input type="checkbox"/>		Suicide	Suicide
<input type="checkbox"/>	<input type="checkbox"/>		Other, _____	Other, _____

FIG. 22A

Have you ever smoked? ☐ Yes ☐ No

If yes, for how long?

0	1
2	3
4	5
6	7
8	9
10	11
12	13
14	15
16	17
18	19
20	21
22	23
24	25
26	27
28	29
30	31
32	33
34	35
36	37
38	39
40	41
42	43
44	45
46	47
48	49
50	51
52	53
54	55
56	57
58	59
60	61
62	63
64	65
66	67
68	69
70	71
72	73
74	75
76	77
78	79
80	81
82	83
84	85
86	87
88	89
90	91
92	93
94	95
96	97
98	99

 Years

Have you been hospitalized in the past 5 years for more than 6 days at a time? ☐ Yes ☐ No

If yes, how many times?

0	1	2	3	4	5	6	7	8	9
---	---	---	---	---	---	---	---	---	---

For each hospitalization (if not the same) how long did you stay and for what reason?

1) Weeks:

0	1	2	3	4	5	6	7	8	9
---	---	---	---	---	---	---	---	---	---

☐ Acute disorder, including infection and thrombosis
☐ Chronic Disorder
☐ Accident
☐ Other: _____

2) Weeks:

0	1	2	3	4	5	6	7	8	9
---	---	---	---	---	---	---	---	---	---

☐ Acute disorder, including infection and thrombosis
☐ Chronic Disorder
☐ Accident
☐ Other: _____

3) Weeks:

0	1	2	3	4	5	6	7	8	9
---	---	---	---	---	---	---	---	---	---

☐ Acute disorder, including infection and thrombosis
☐ Chronic Disorder
☐ Accident
☐ Other: _____

Have you or has anyone in your immediate family (parents, brothers, sisters, or your children) had the following? Mark all that apply!

Disease	You	Mother	Father	Sister	Brother	Child
Heart Disease, including arteriosclerosis	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Stroke	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Hypertension	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Blood clots	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Diabetes, insulin dependent	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Diabetes, not insulin-dependent (diet controlled)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Cancer:	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Lung&Bronchus	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Breasts	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Prostate	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Colon&Rectum	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Skin	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Lymphoma&Leukemia	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Other, please specify below:	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Alzheimer's Disease	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Epilepsy	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Schizophrenia	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Bipolar disorder (manic depression)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Major depression	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Chronic Inflammatory or Autoimmune Disease including Multiple Sclerosis and Rheumatoid Arthritis	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Emphysema	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Asthma	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Other, please specify below:	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Do you take prescription drugs on a regular basis? ☐ Yes ☐ No

If yes, please specify below:

Have you ever donated blood before? ☐ Yes ☐ No

If yes, how many times:

0	1	2	3	4	5	6	7	8	9
---	---	---	---	---	---	---	---	---	---

 Number of Times

Additional health information details you would like to provide:

FOR OFFICE USE ONLY

Do you drink any kind of alcoholic beverage?

☐ Never ☐ Hardly ever
☐ Less than 3 times per week ☐ 3 or more times per week
☐ Daily

FIG. 22B

Collection Information

Consent Form Signed

☐ Yes
☐ No

Date of Collection

Month

Day

Year

Time of Sample Collection

(nearest hour in 24-hour clock format)

Initials

Initials of Data Collector

(DO NOT COMPLETE for data entry only)

Sample:

☐ Intact
☐ Lost
☐ Broken

Volume (ml)

Donor Information

Date of Birth

Month

Year

Sex:

☐ Male
☐ Female

Height

Ft. Inches

Weight (lb)

What physical activity do you do on a regular basis?

☐ Running
☐ Swimming
☐ Biking
☐ Gymnastics
☐ Other
☐ None

Are you a vegetarian?

☐ Yes
☐ No

If female:

How many times have you been pregnant?

How many times did you give birth?

In which state do you live?

To the best of your knowledge, what is the Ethnic Origin of your:

Father:

Mother:

☐ Caucasian (please mark specific geographic area below if known)
☐ Northern Europe (Austria, Denmark, Finland, France, Germany, Netherlands, Norway, Sweden, Switzerland, UK)
☐ Southern Europe (Greece, Italy, Spain, Turkey)
☐ Eastern Europe (Czechoslovakia, Hungary, Poland, Russia, Yugoslavia)
☐ Middle Eastern (Israel, Egypt, Iran, Iraq, Jordan, Syria, Other Arab States)
☐ Other
☐ Don't know

How long have you lived there?

Years

How many years have you been smoking?

Years

Did you quit smoking?

☐ Yes
☐ No

If yes, how many years ago?

Years

How many cigarettes do/did you smoke per day?

Years

Do you have lung Emphysema?

☐ Yes
☐ No

If yes, for how long?

Years

Continue on back

FIG. 22C

What is your highest grade you completed in school?
☐ less than 8th grade
☐ 8th, 9th, 10th, or 11th grade
☐ high school graduate or equivalency
☐ some college, 2yr degree
☐ college graduate, 4yr degree
☐ post graduate education or degree

Mother Deceased? Cause of Death Mother: ☐ Yes ☐ No
 If Yes at what age?
☐ < 29 ☐ Heart Disease
☐ 30-39 ☐ Cancer
☐ 40-49 ☐ Stroke
☐ 50-59 ☐ Accident
☐ 60-69 ☐ Suicide
☐ 70-79 ☐ Other, _____
☐ 80-89
☐ ≥ 90

Father Deceased? Cause of Death Father: ☐ Yes ☐ No
 If Yes at what age?
☐ < 29 ☐ Heart Disease
☐ 30-39 ☐ Cancer
☐ 40-49 ☐ Stroke
☐ 50-59 ☐ Accident
☐ 60-69 ☐ Suicide
☐ 70-79 ☐ Other, _____
☐ 80-89
☐ > 90

Health Information

Have you or has anyone in your immediate family (parents, brothers, sisters, or your children) had the following?

Mark all that apply!

Disease	You	Mother	Father	Sister	Brother	Child
Heart Disease	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Stroke	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Hypertension	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Blood clots	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Diabetes, insulin dependent	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Diabetes, not insulin-dependent	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Cancer:	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Lung & Bronchus	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Breasts	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Prostate	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Colon & Rectum	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Skin	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Lymphoma & Leukemia	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Other, please specify below:	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Alzheimer's Disease	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Epilepsy	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Schizophrenia	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Bipolar disorder (manic depression)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Major depression	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Chronic Inflammatory or Autoimmune Disease including Multiple Sclerosis and Rheumatoid Arthritis	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Emphysema	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Asthma	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Other, please specify below:	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Do you take prescription drugs on a regular basis?

☐ Yes ☐ No

Have you ever donated blood before? ☐ Yes ☐ No

If yes, please specify below:

If yes, how many times: Number of Times

Have you been hospitalized in the past 5 years for more than 6 days at a time?
☐ Yes ☐ No

If yes, how many times?
☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9

For each hospitalization (if not the same) how long did you stay and for what reason?

1) Weeks: ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6
☐ Acute disorder, including infection and thrombosis
☐ Chronic Disorder
☐ Accident
☐ Other: _____

2) Weeks: ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6
☐ Acute disorder, including infection and thrombosis
☐ Chronic Disorder
☐ Accident
☐ Other: _____

3) Weeks: ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6
☐ Acute disorder, including infection and thrombosis
☐ Chronic Disorder
☐ Accident
☐ Other: _____

<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Do you drink any kind of alcoholic beverage?

☐ Never ☐ Hardly ever
☐ Less than 3 times per week ☐ 3 or more times per week
☐ Daily

Additional health information details you would like to provide:

FOR OFFICE USE ONLY

<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>

FIG. 22D

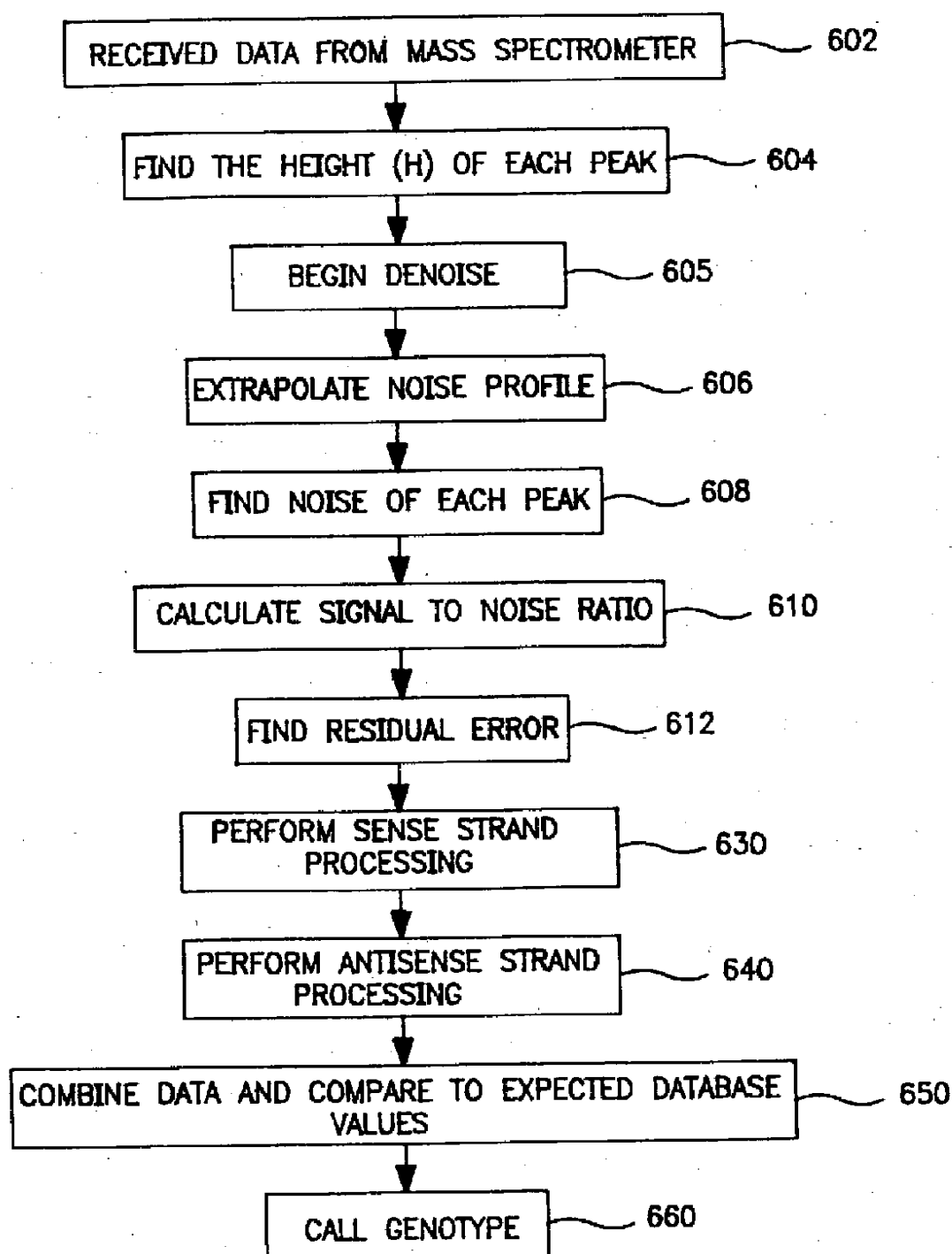


FIG. 23

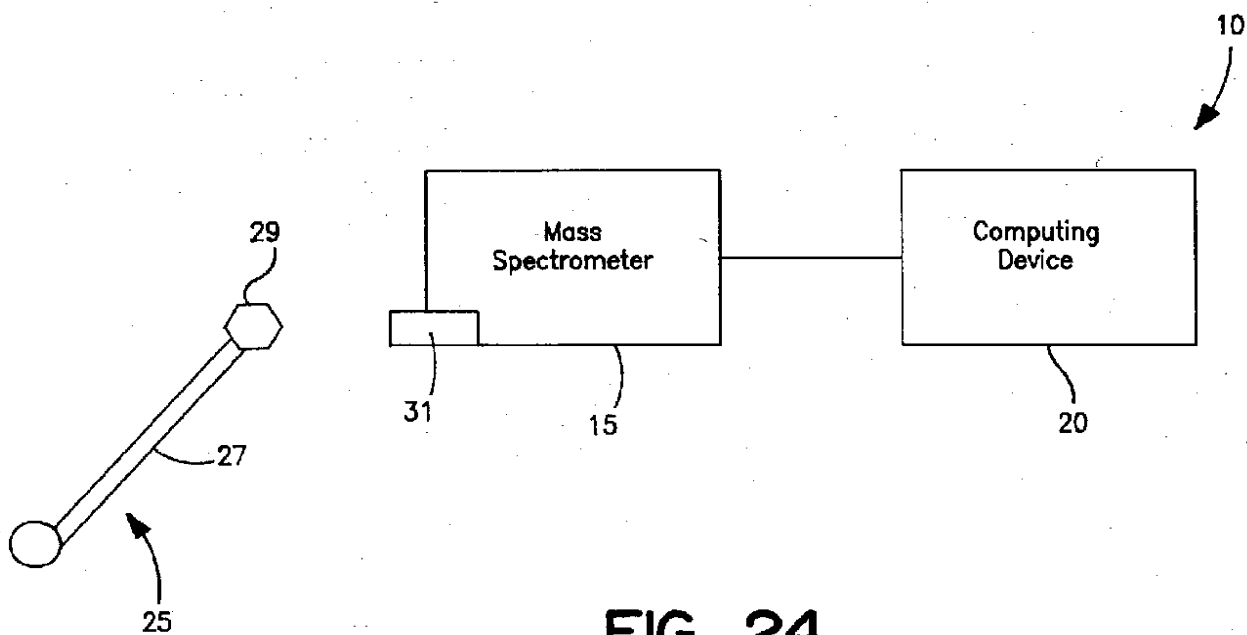


FIG. 24

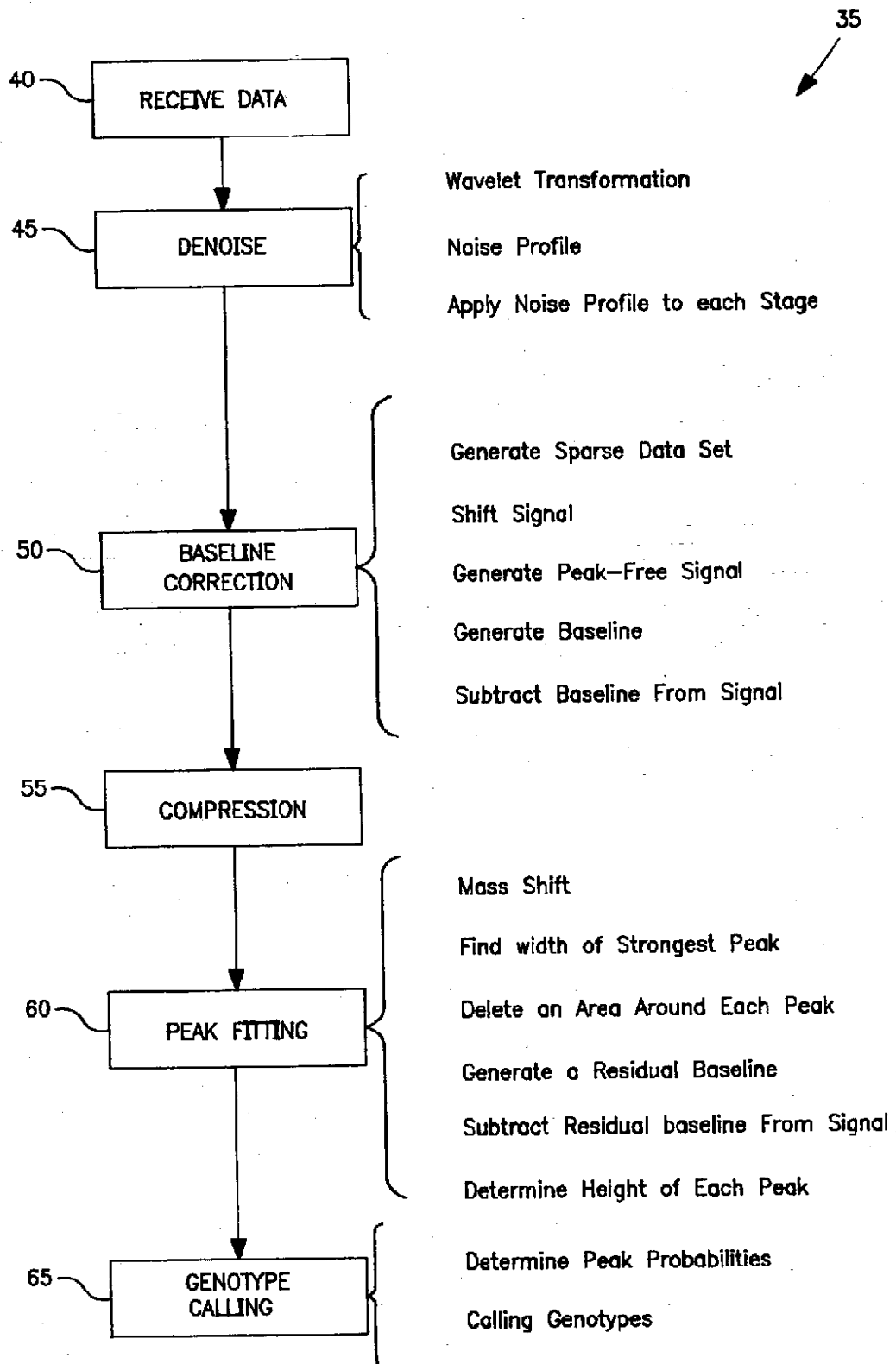


FIG. 25

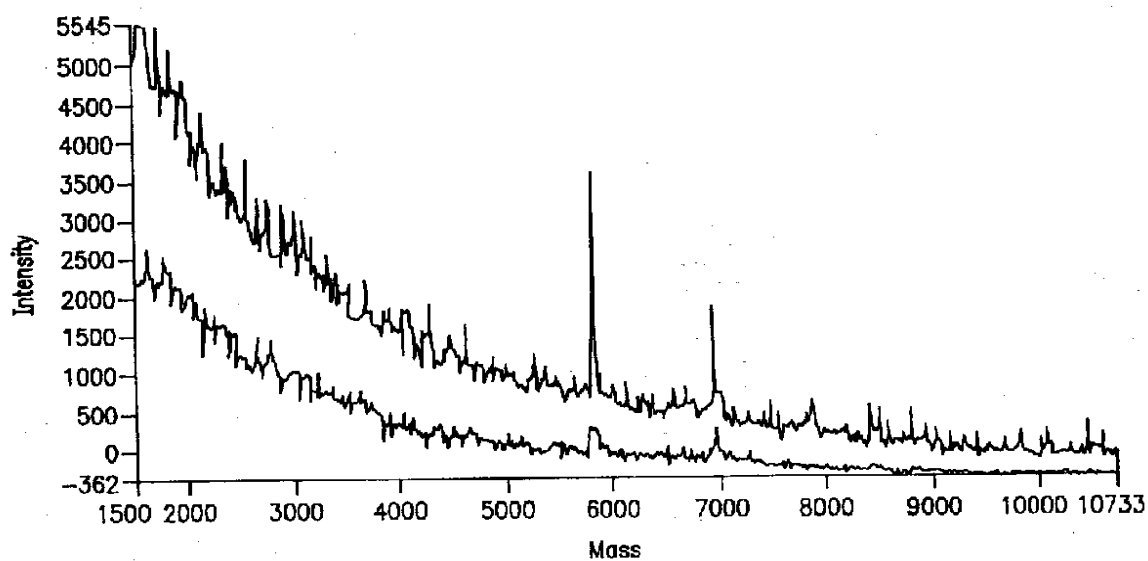


FIG. 26

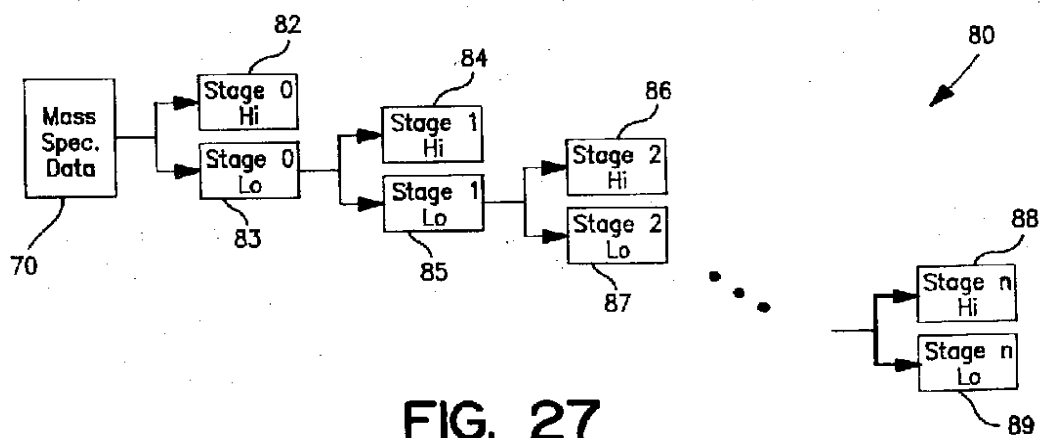


FIG. 27

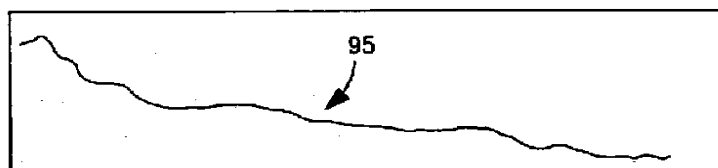


FIG. 28

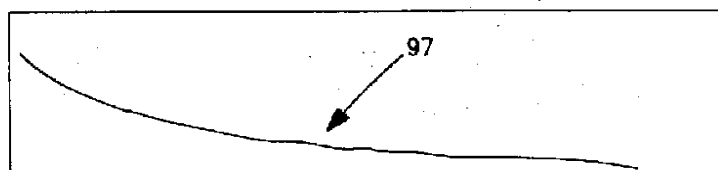


FIG. 29

Exp fitting
 $a_0 + a_1 \exp^{-a_2 m}$

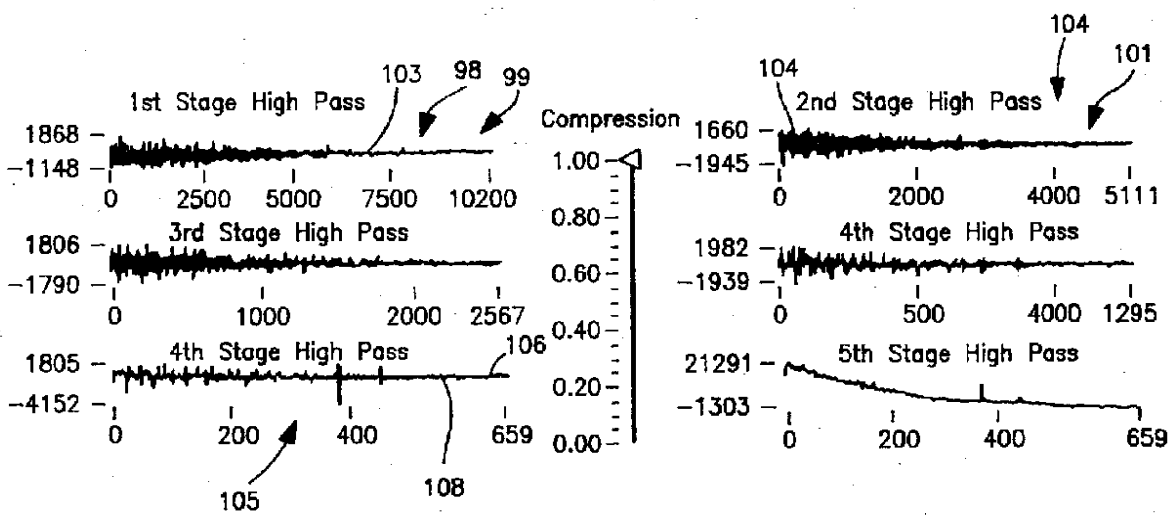
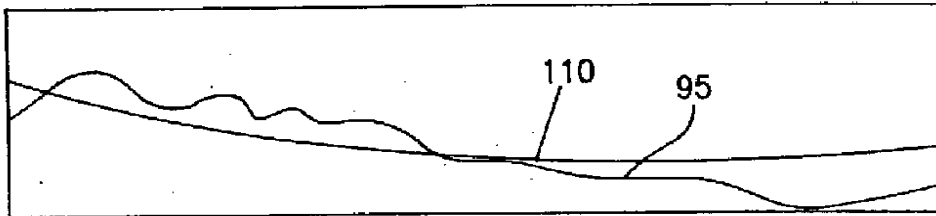


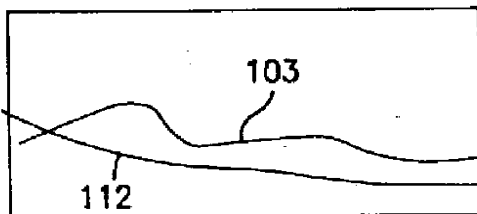
FIG. 30

Stage 0 - Hi



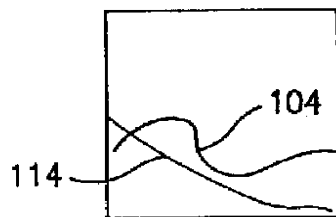
Threshold 0=4XNoiseProfile

Stage 1 - Hi



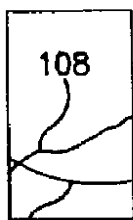
Threshold 1=2XNoiseProfile

Stage 2 - Hi



Threshold 2=1XNoiseProfile

Stage n - Hi



Threshold $n=(1/2^{n-2})\text{XNoiseProfile}$

116

Stage n - Lo



118

FIG. 3I

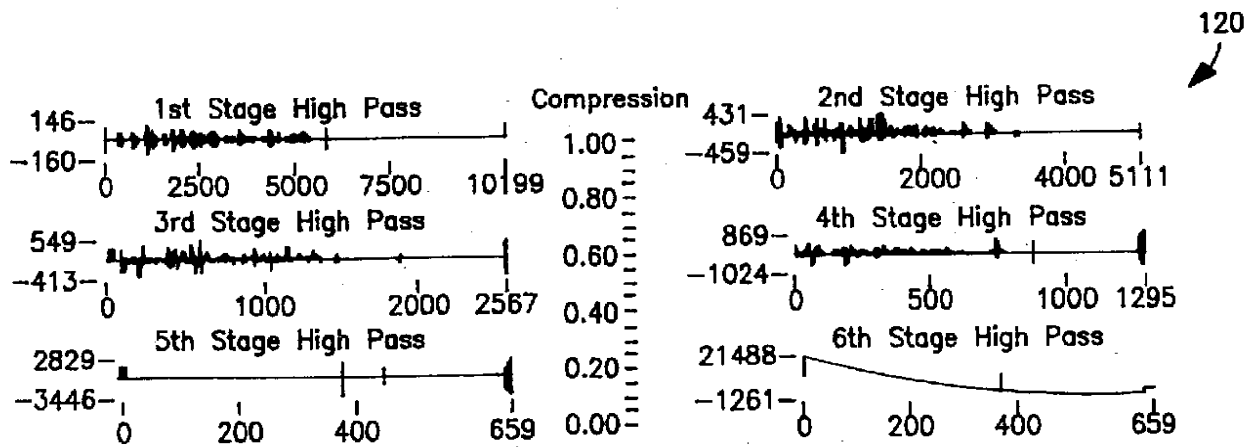


FIG. 32

$$\text{Signal (t)} = \frac{(\text{Start 0(t)} + \text{Start 1(t)} + \text{Start 2(t)} \dots + \text{Start 23 (t)})}{24}$$

SHIFT SIGNAL TO ACCOUNT FOR
VARIATIONS DUE TO STARTING POINT

FIG. 33

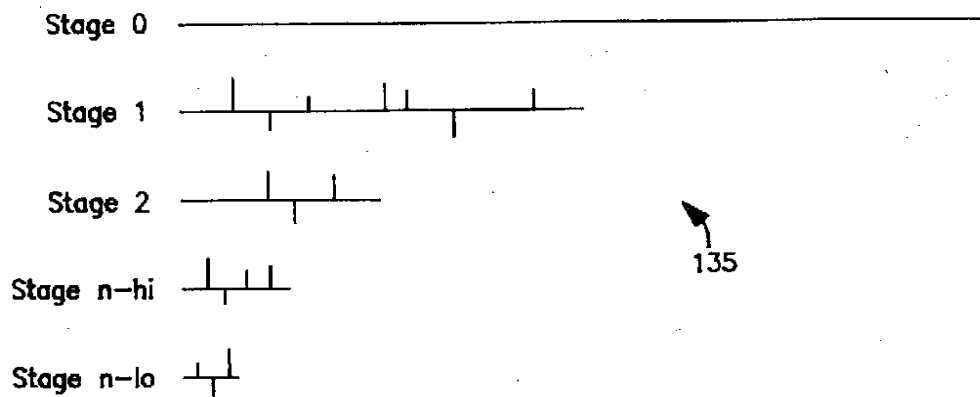


FIG. 34

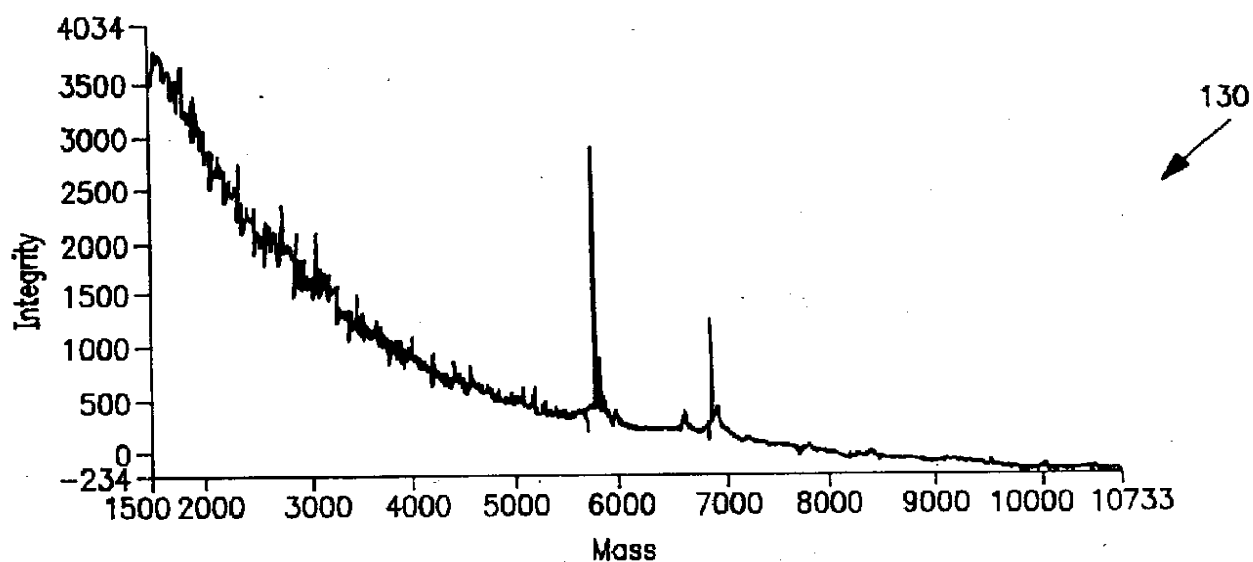


FIG. 35

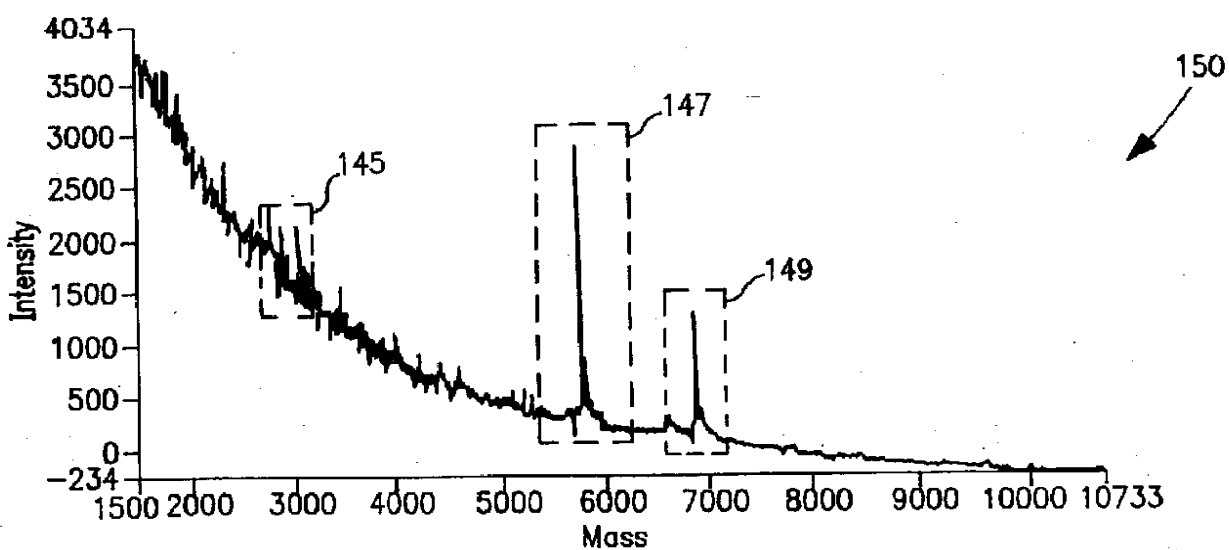


FIG. 13—TAKE A MOVING AVERAGE, REMOVE SECTIONS EXCEEDING A THRESHOLD

FIG. 36

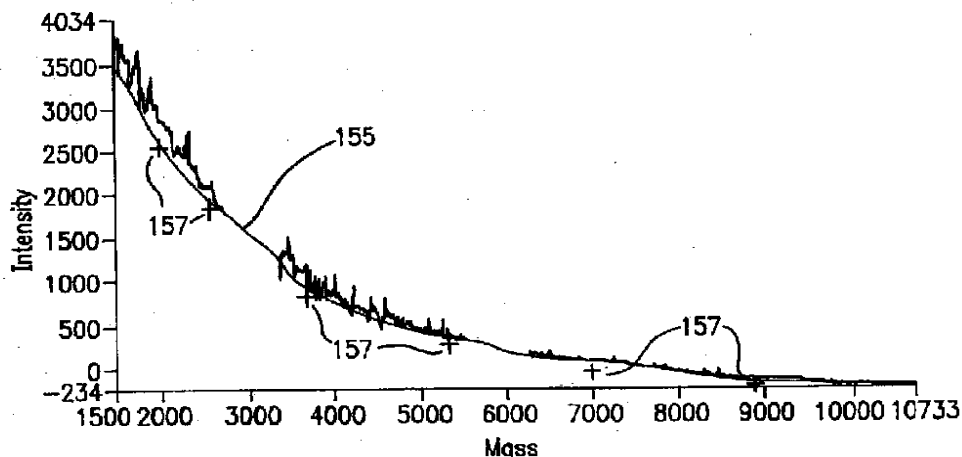


FIG. 37

FIND MINIMA IN REMAINING SIGNALS AND CONNECT TO FORM A PEAK FREE SIGNAL

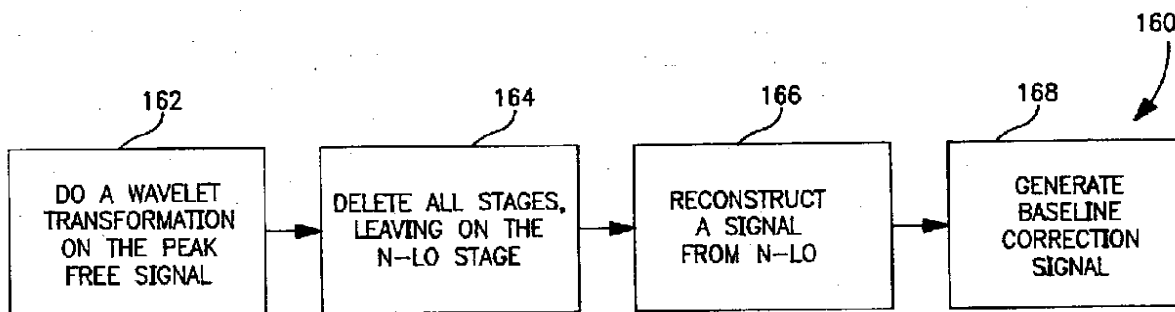


FIG. 38

GENRATE BASELINE CORRECTION

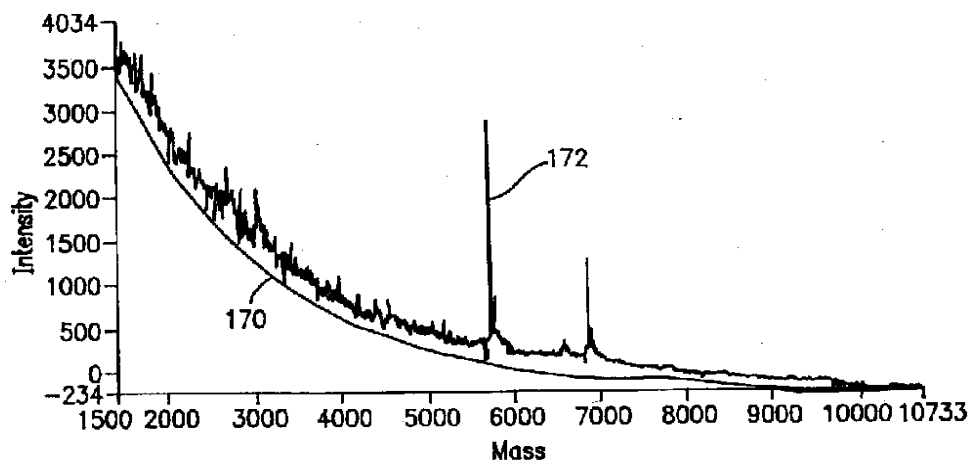


FIG. 39

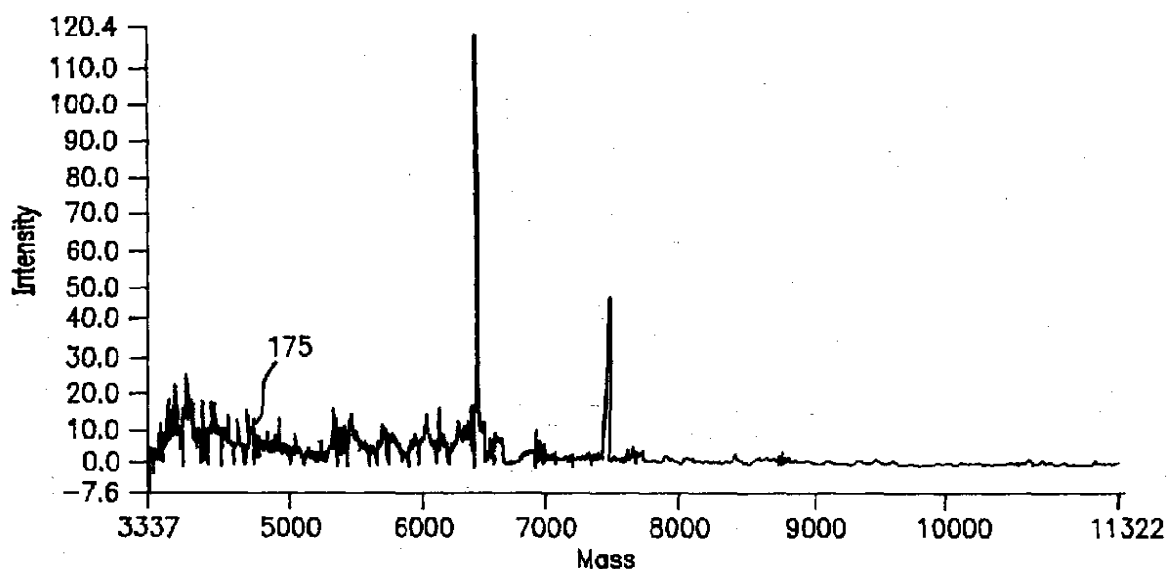


FIG. 40

NON-O COEFFICIENTS	COEFFICIENTS	INTERMEDIATE	RELATIVE
<div>183</div> 100 150 500 10,050 10,075 11,125 12,100 13,250	<div>182</div> 25 220 .1 800 890 910 1000 (MAX) 940	<div>186</div> 100.025 150.220 500.0001 10,050.8 10,075.89 11,125.91 12,100.99999 13,250.94	<div>180</div> <div>195</div> 100.025 50.220 350.0001 9550.8 25.89 150.91 975.99999 1150.94

FIG. 41

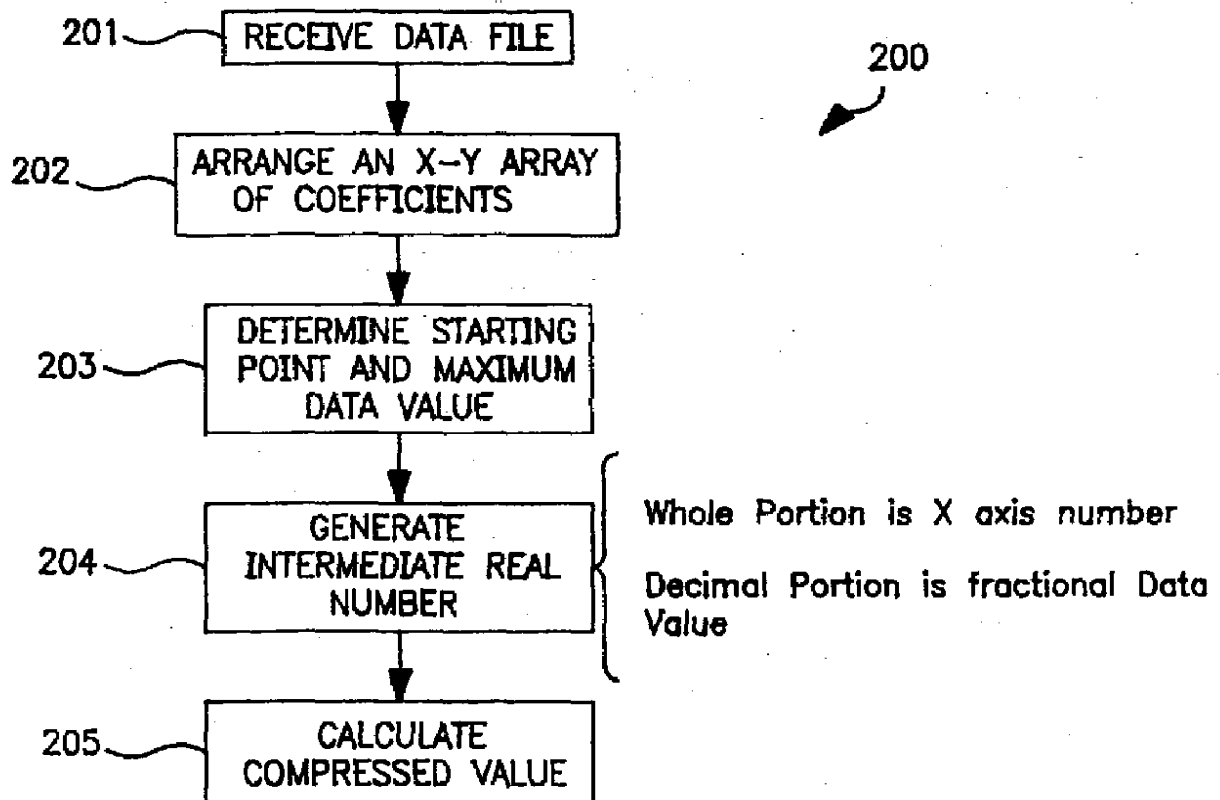


FIG. 42

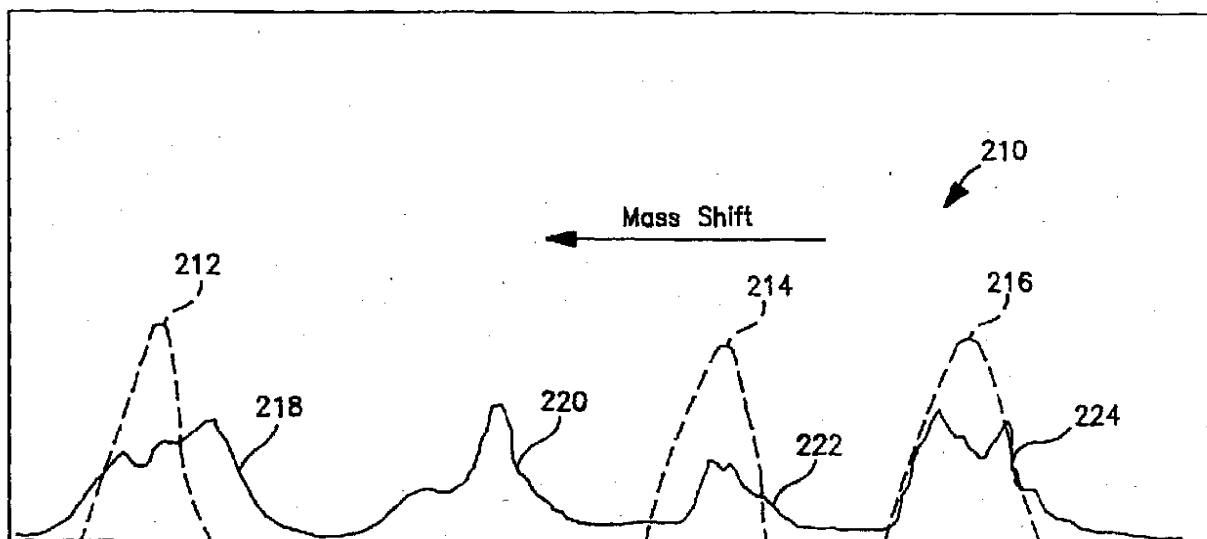


FIG. 43

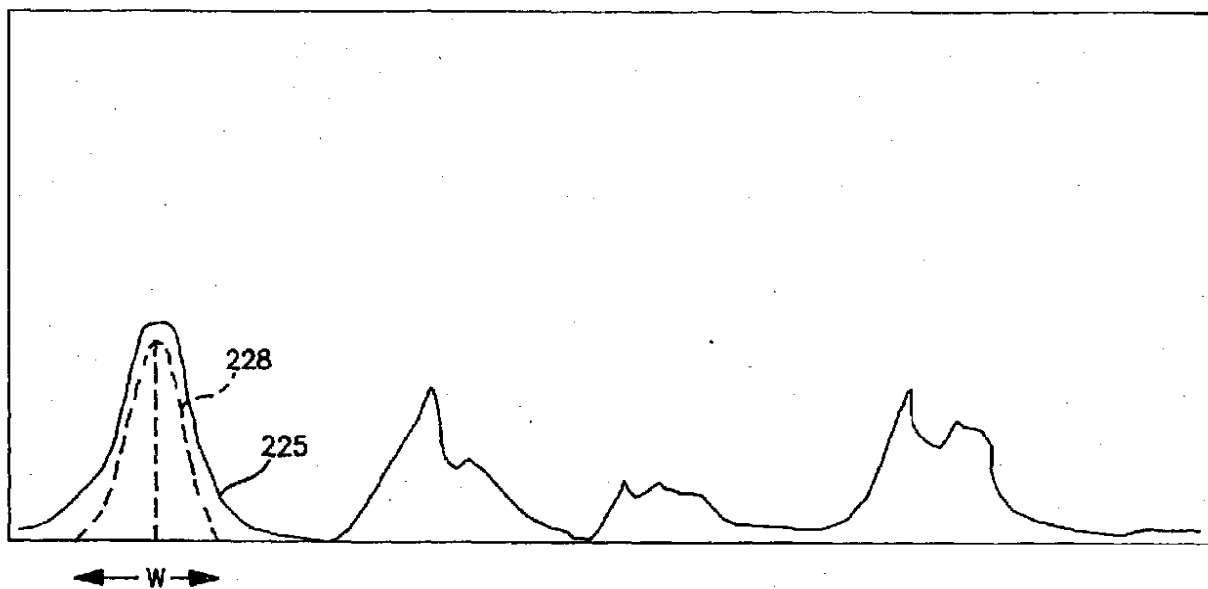


FIG. 44

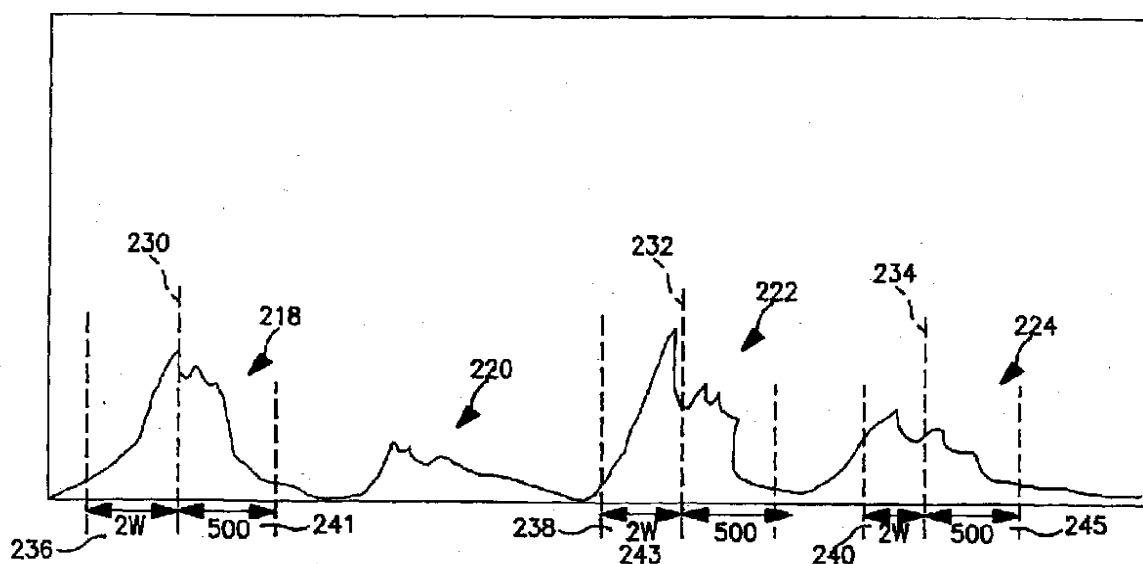


FIG. 45

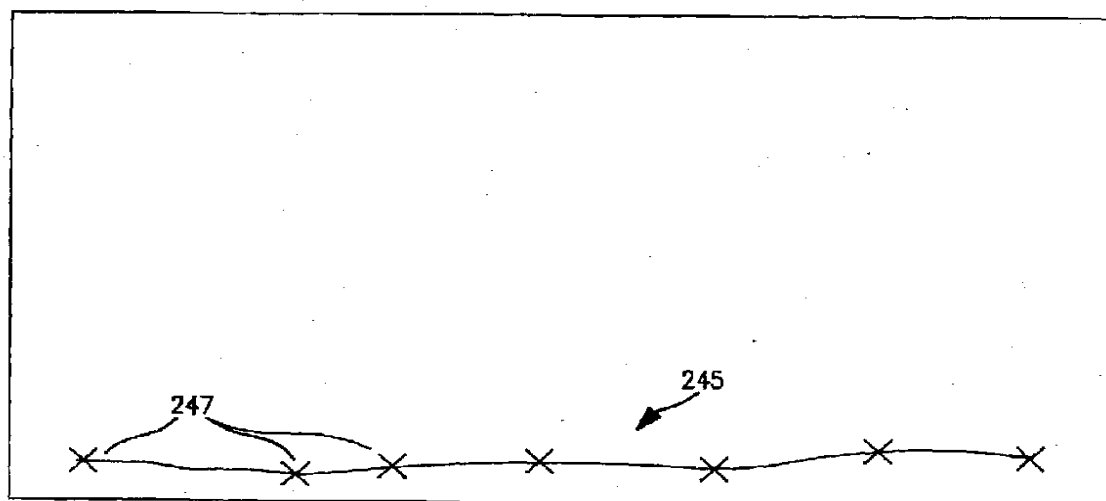


FIG. 46

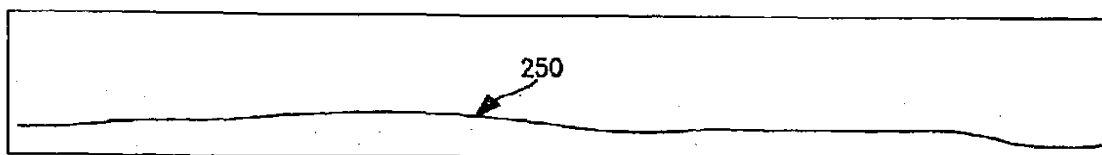


FIG. 47

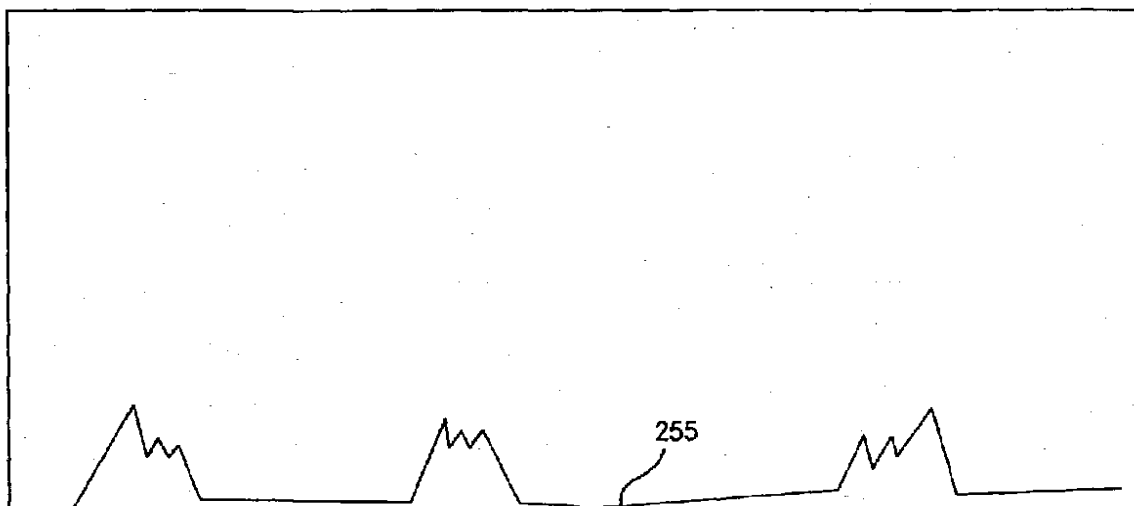


FIG. 48

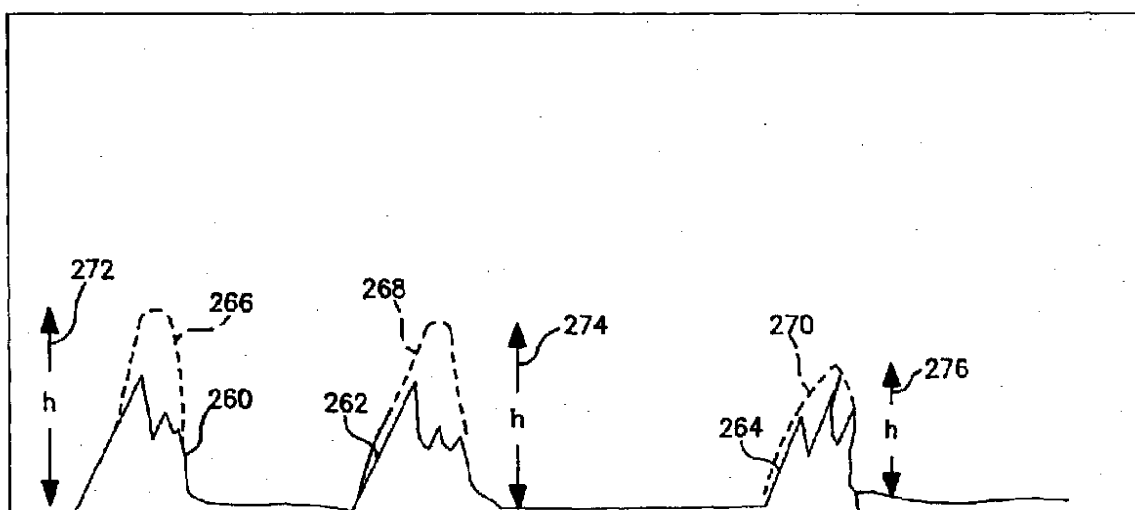


FIG. 49

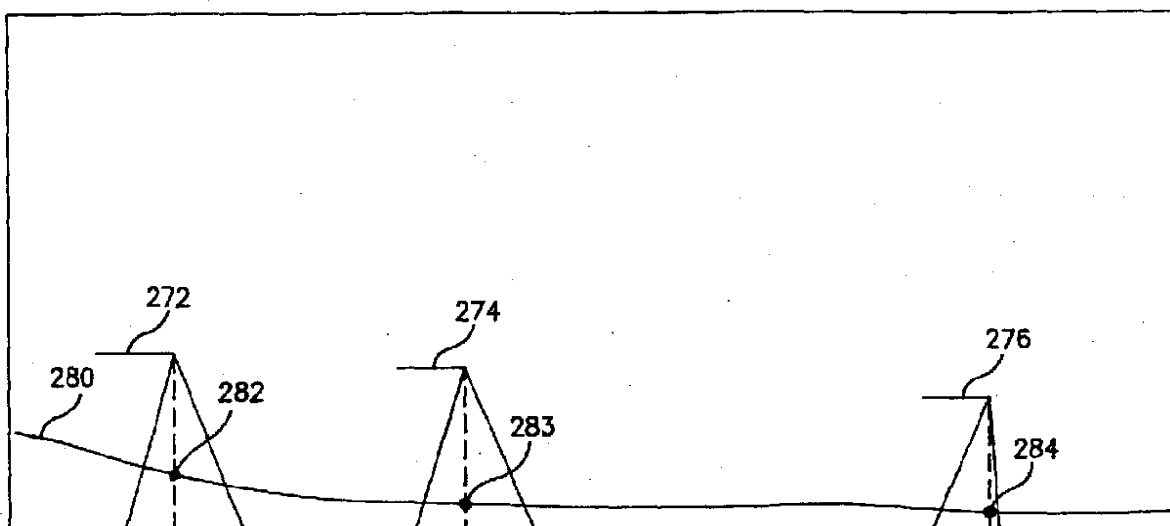


FIG. 50

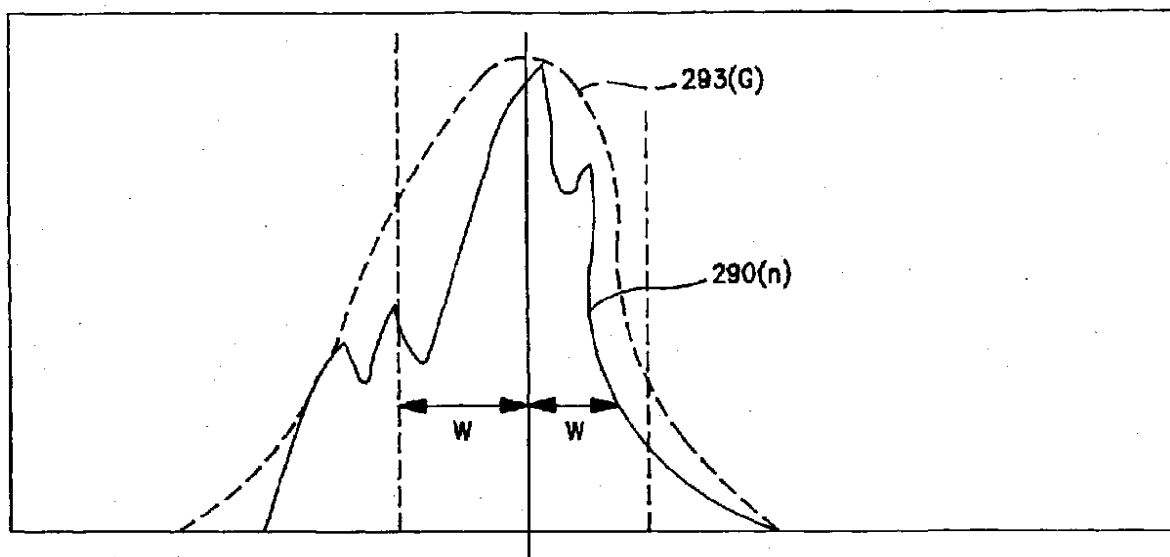
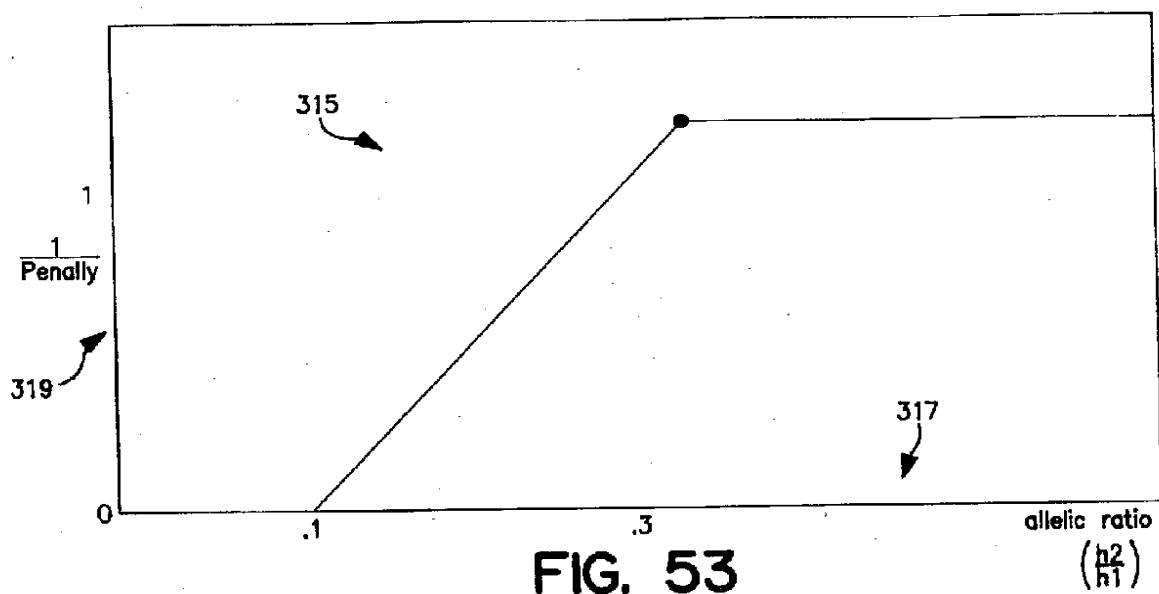
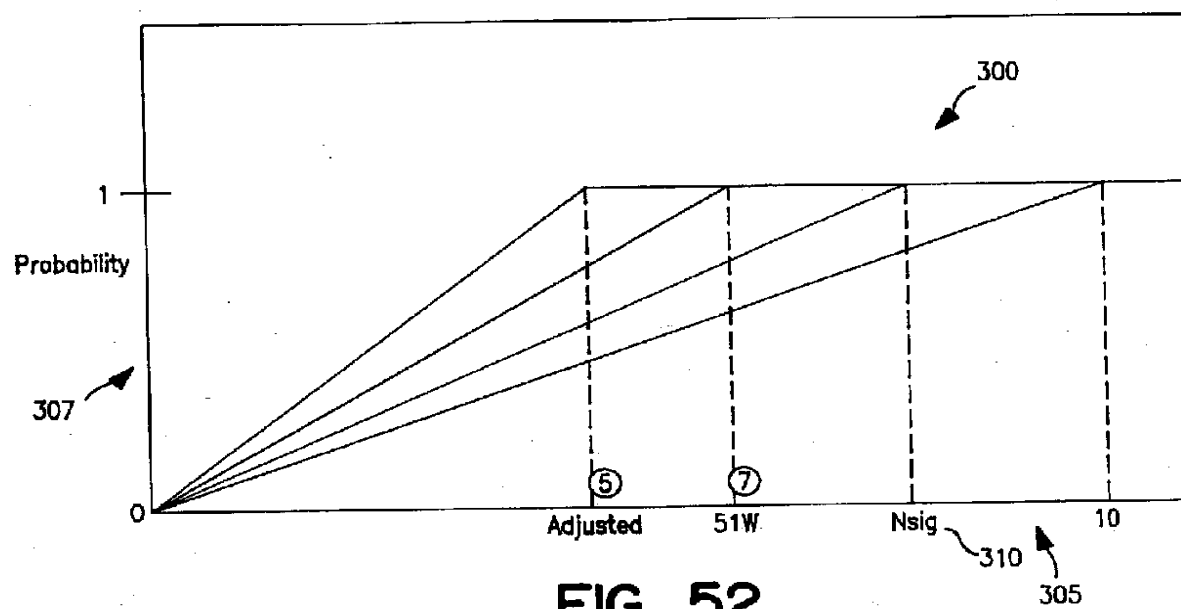


FIG. 51



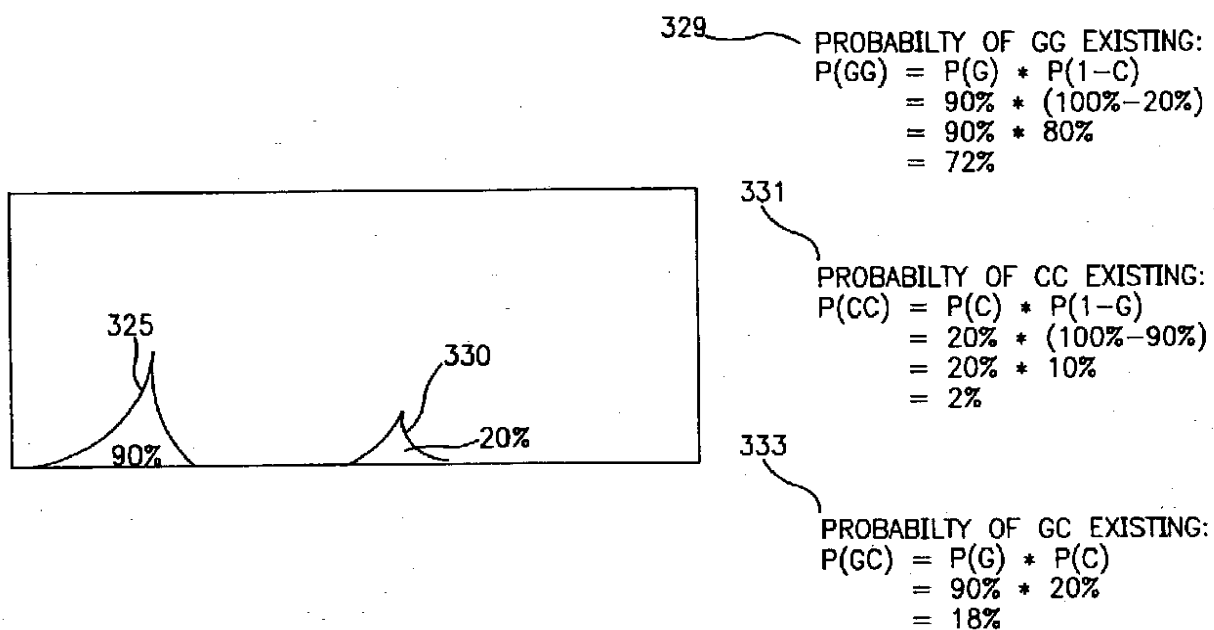


FIG. 54

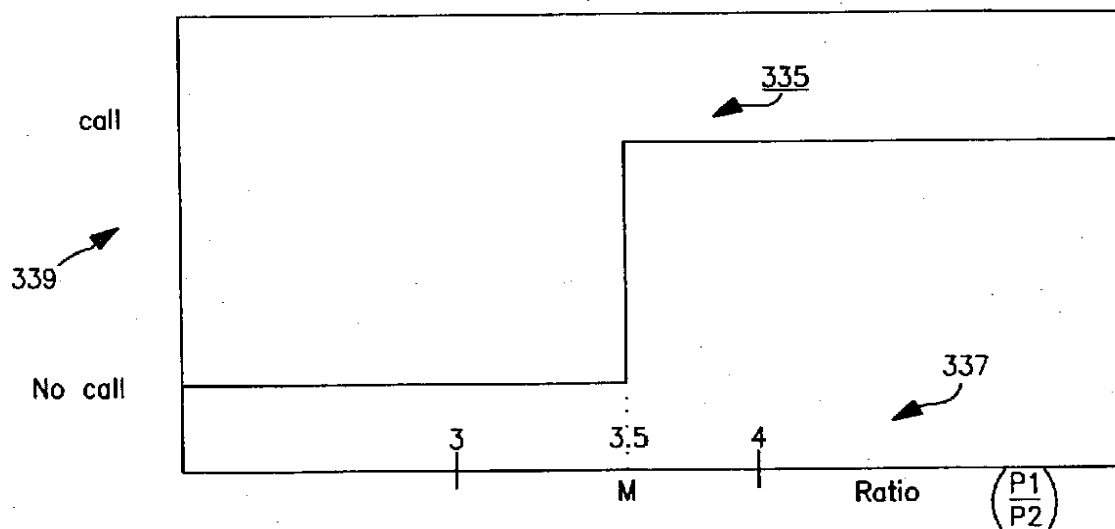


FIG. 55

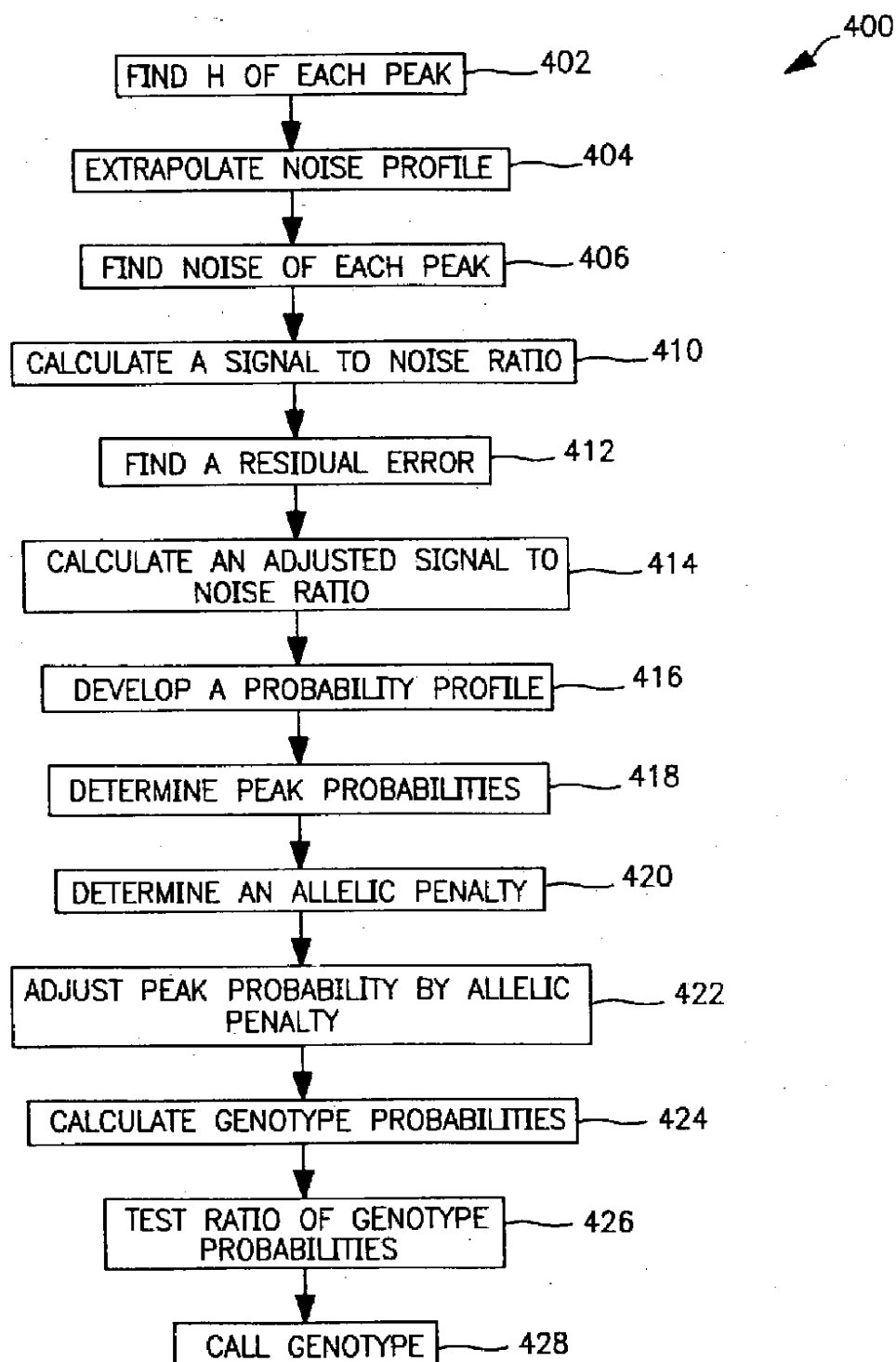


FIG. 56

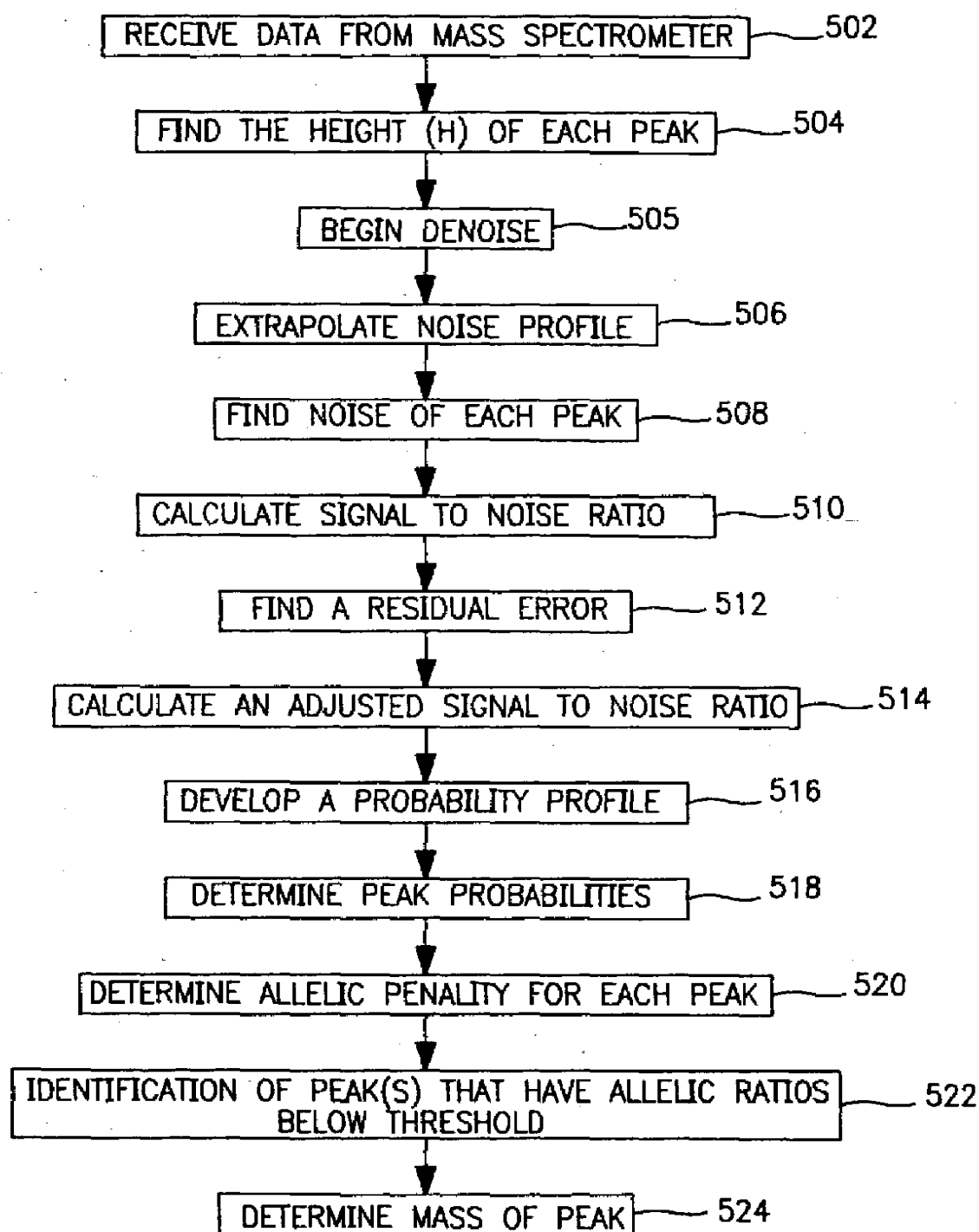


FIG. 57

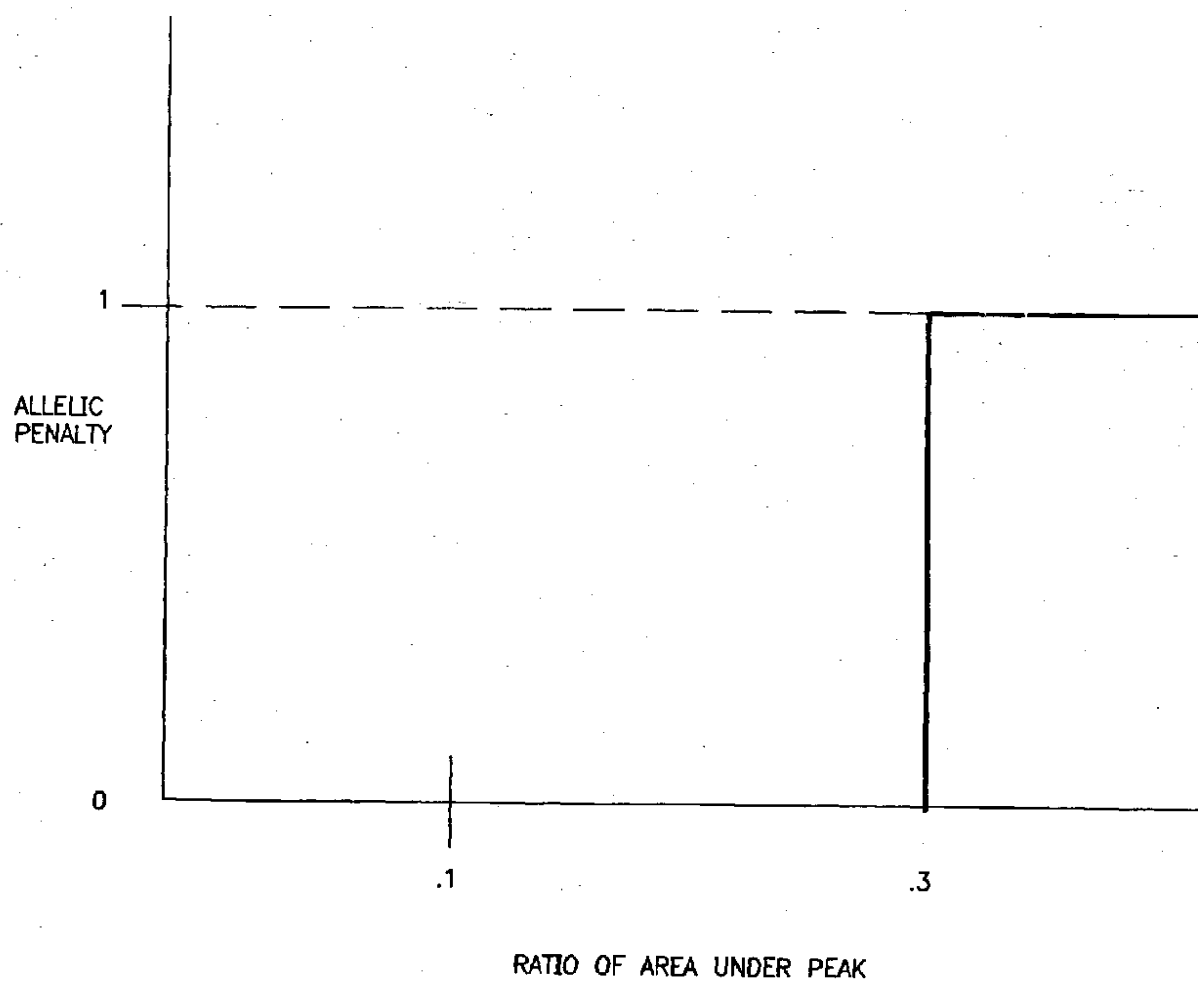


FIG. 58

METHODS FOR GENERATING DATABASES AND DATABASES FOR IDENTIFYING POLYMORPHIC GENETIC MARKERS

RELATED APPLICATIONS

[0001] This application is a divisional application of copending U.S. patent application Ser. No. 09/687,483, filed Oct. 13, 2000, to Andreas Braun, Hubert Koster, Dirk Van den Boom, Yip Ping, Charles Rodi, Liyan He, Norman Chiu and Christian Jurinke entitled "METHODS FOR GENERATING DATABASES AND DATABASES FOR IDENTIFYING POLYMORPHIC GENETIC MARKERS."

[0002] Benefit of priority under 35 U.S.C. §119(e) to the following provisional applications is claimed herein: U.S. provisional application Serial No. 60/217,658 to Andreas Braun, Hubert Koster, Dirk Van den Boom, filed Jul. 10, 2000, entitled "METHODS FOR GENERATING DATABASES AND DATABASES FOR IDENTIFYING POLYMORPHIC GENETIC MARKERS"; U.S. provisional application Serial No. 60/159,176 to Andreas Braun, Hubert Koster, Dirk Van den Boom, filed Oct. 13, 1999, entitled "METHODS FOR GENERATING DATABASES AND DATABASES FOR IDENTIFYING POLYMORPHIC GENETIC MARKERS"; U.S. provisional application Serial No. 60/217,251, filed Jul. 10, 2000, to Andreas Braun, entitled "POLYMORPHIC KINASE ANCHOR PROTEIN GENE SEQUENCES, POLYMORPHIC KINASE ANCHOR PROTEINS AND METHODS OF DETECTING POLYMORPHIC KINASE ANCHOR PROTEINS AND NUCLEIC ACIDS ENCODING THE SAME". This application is also a continuation-in-part of U.S. application Serial No. 09/663,968, to Ping Yip, filed Sep. 19, 2000, entitled "METHOD AND DEVICE FOR IDENTIFYING A BIOLOGICAL SAMPLE."

[0003] The above-noted applications and provisional applications are incorporated by reference in their entirety.

FIELD OF THE INVENTION

[0004] Process and methods for creating a database of genomic samples from healthy human donors. Methods that use the database to identify and correlate with polymorphic genetic markers and other markers with diseases and conditions are provided.

BACKGROUND

[0005] Diseases in all organisms have a genetic component, whether inherited or resulting from the body's response to environmental stresses, such as viruses and toxins. The ultimate goal of ongoing genomic research is to use this information to develop new ways to identify, treat and potentially cure these diseases. The first step has been to screen disease tissue and identify genomic changes at the level of individual samples. The identification of these "disease" markers has then fueled the development and commercialization of diagnostic tests that detect these errant genes or polymorphisms. With the increasing numbers of genetic markers, including single nucleotide polymorphisms (SNPs), microsatellites, tandem repeats, newly mapped introns and exons, the challenge to the medical and pharmaceutical communities is to identify genotypes which not only identify the disease but also follow the progression of the disease and are predictive of an organism's response to treatment.

[0006] Currently the pharmaceutical and biotechnology industries find a disease and then attempt to determine the genomic basis for the disease. This approach is time consuming and expensive and in many cases involves the investigator guessing as to what pathways might be involved in the disease.

[0007] Genomics

[0008] Presently the two main strategies employed in analyzing the available genomic information are the technology driven reverse genetics brute force strategy and the knowledge-based pathway oriented forward genetics strategy. The brute force approach yields large databases of sequence information but little information about the medical or other uses of the sequence information. Hence this strategy yields intangible products of questionable value. The knowledge-based strategy yields small databases that contain a lot of information about medical uses of particular DNA sequences and other products in the pathway and yield tangible products with a high value.

[0009] Polymorphisms

[0010] Polymorphisms have been known since 1901 with the identification of blood types. In the 1950's they were identified on the level of proteins using large population genetic studies. In the 1980's and 1990's many of the known protein polymorphisms were correlated with genetic loci on genomic DNA. For example, the gene dose of the apolipoprotein E type 4 allele was correlated with the risk of Alzheimer's disease in late onset families (see, e.g., Corder et al. (1993) *Science* 261: 921-923; mutation in blood coagulation factor V was associated with resistance to activated protein C (see, e.g., Bertina et al. (1994) *Nature* 369:64-67); resistance to HIV-1 infection has been shown in Caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene (see, e.g., Samson et al. (1996) *Nature* 382:722-725); and a hypermutable tract in antigen presenting cells (APC, such as macrophages), has been identified in familial colorectal cancer in individuals of Ashkenzi jewish background (see, e.g., Laken et al. (1997) *Nature Genet.* 17:79-83). There can be more than three million polymorphic sites in the human genome. Many have been identified, but not yet characterized or mapped or associated with a marker.

[0011] Single Nucleotide Polymorphisms (SNPs)

[0012] Much of the focus of genomics has been in the identification of SNPs, which are important for a variety of reasons. They allow indirect testing (association of haplotypes) and direct testing (functional variants). They are the most abundant and stable genetic markers. Common diseases are best explained by common genetic alterations, and the natural variation in the human population aids in understanding disease, therapy and environmental interactions.

[0013] Currently, the only available method to identify SNPs in DNA is by sequencing, which is expensive, difficult and laborious. Furthermore, once a SNP is discovered it must be validated to determine if it is a real polymorphism and not a sequencing error. Also, discovered SNPs must then be evaluated to determine if they are associated with a particular phenotype. Thus, there is a need to develop new paradigms for identifying the genomic basis for disease and markers thereof. Therefore, it is an object herein to provide methods for identifying the genomic basis of disease and markers thereof.

SUMMARY

[0014] Databases and methods using the databases are provided herein. The databases comprise sets of parameters associated with subjects in populations selected only on the basis of being healthy (i.e., where the subjects are mammals, such as humans, they are selected based upon apparent health and no detectable infections). The databases can be sorted based upon one or more of the selected parameters.

[0015] The databases, for example, can be relational databases, in which an index that represents each subject serves to relate parameters, which are the data, such as age, ethnicity, sex, medical history, etc. and ultimately genotypic information, that was inputted into and stored in the database. The database can then be sorted according to these parameters. Initially, the parameter information is obtained from a questionnaire answered by each subject from whom a body tissue or body fluid sample is obtained. As additional information about each sample is obtained, this information can be entered into the database and can serve as a sorting parameter.

[0016] The databases obtained from healthy individuals have numerous uses, such as correlating known polymorphisms with a phenotype or disease. The databases can be used to identify alleles that are deleterious, that are beneficial, and that are correlated with diseases.

[0017] For purposes herein, genotypic information can be obtained by any method known to those of skill in the art, but is generally obtained using mass spectrometry.

[0018] Also provided herein, is a new use for existing databases of subjects and genotypic and other parameters, such as age, ethnicity, race, and gender. Any database can be sorted according to the methods herein, and alleles that exhibit statistically significant correlations with any of the sorting parameters can be identified. It is noted, however, is noted, that the databases provided herein and randomly selected databases will perform better in these methods, since disease-based databases suffer numerous limitations, including their relatively small size, the homogeneity of the selected disease population, and the masking effect of the polymorphism associated with the markers for which the database was selected. Hence, the healthy database provided herein, provides advantages not heretofore recognized or exploited. The methods provided herein can be used with a selected database, including disease-based databases, with or without sorting for the discovery and correlation of polymorphisms. In addition, the databases provided herein represent a greater genetic diversity than the unselected databases typically utilized for the discovery of polymorphisms and thus allow for the enhanced discovery and correlation of polymorphisms.

[0019] The databases provided herein can be used for taking an identified polymorphism and ascertaining whether it changes in frequency when the data are sorted according to a selected parameter.

[0020] One use of these methods is correlating a selected marker with a particular parameter by following the occurrence of known genetic markers and then, having made this correlation, determining or identifying correlations with diseases. Examples of this use are p53 and Lipoprotein Lipase polymorphism. As exemplified herein, known markers are shown to have particular correlation with certain

groups, such as a particular ethnicity or race or one sex. Such correlations will then permit development of better diagnostic tests and treatment regimens.

[0021] These methods are valuable for identifying one or more genetic markers whose frequency changes within the population as a function of age, ethnic group, sex or some other criteria. This can allow the identification of previously unknown polymorphisms and ultimately a gene or pathway involved in the onset and progression of disease.

[0022] The databases and methods provided herein permit, among other things, identification of components, particularly key components, of a disease process by understanding its genetic underpinnings and also permit an understanding of processes, such as individual drug responses. The databases and methods provided herein also can be used in methods involving elucidation of pathological pathways, in developing new diagnostic assays, identifying new potential drug targets, and in identifying new drug candidates.

[0023] The methods and databases can be used with experimental procedures, including, but are not limited to, in silico SNP identification, in vitro SNP identification/verification, genetic profiling of large populations, and in bio-statistical analyses and interpretations.

[0024] Also provided herein, are combinations that contain a database provided herein and a biological sample from a subject in the database, and typically biological samples from all subjects or a plurality of subjects in the database. Collections of the tissue and body fluid samples are also provided.

[0025] Also, provided herein, are methods for determining a genetic marker that correlates with age, comprising identifying a polymorphism and determining the frequency of the polymorphism with increasing age in a healthy population.

[0026] Further provided herein are methods for determining whether a genetic marker correlates with susceptibility to morbidity, early mortality, or morbidity and early mortality, comprising identifying a polymorphism and determining the frequency of the polymorphism with increasing age in a healthy population.

[0027] Any of the methods herein described can be used out in a multiplex format.

[0028] Also provided are an apparatus and process for accurately identifying genetic information. It is another object herein that genetic information be extracted from genetic data in a highly automated manner. Therefore, to overcome the deficiencies in the known conventional systems, methods and apparatus for identifying a biological sample are provided.

[0029] Briefly, the method and system for identifying a biological sample generates a data set indicative of the composition of the biological sample. In a particular example, the data set is DNA spectrometry data received from a mass spectrometer. The data set is denoised, and a baseline is deleted. Since possible compositions of the biological sample can be known, expected peak areas can be determined. Using the expected peak areas, a residual baseline is generated to further correct the data set. Probable peaks are then identifiable in the corrected data set, which are used to identify the composition of the biological

sample. In a disclosed example, statistical methods are employed to determine the probability that a probable peak is an actual peak, not an actual peak, or that the data too inconclusive to call.

[0030] Advantageously, the method and system for identifying a biological sample accurately makes composition calls in a highly automated manner. In such a manner, complete SNP profile information, for example, can be collected efficiently. More importantly, the collected data are analyzed with highly accurate results. For example, when a particular composition is called, the result can be relied upon with great confidence. Such confidence is provided by the robust computational process employed.

DESCRIPTION OF THE DRAWINGS

[0031] FIG. 1 depicts an exemplary sample bank. Panel 1 shows the samples as a function of sex and ethnicity. Panel 2 shows the Caucasians as a function of age. Panel 3 shows the Hispanics as a function of age.

[0032] FIGS. 2A and 2C show an age- and sex-distribution of the 291S allele of the lipoprotein lipase gene in which a total of 436 males and 589 females were investigated. FIG. 2B shows an age distribution for the 436 males.

[0033] FIG. 3 is an exemplary questionnaire for population-based sample banking.

[0034] FIG. 4 depicts processing and tracking of blood sample components.

[0035] FIG. 5 depicts the allelic frequency of "sick" alleles and "healthy" alleles as a function of age. It is noted that the relative frequency of healthy alleles increases in a population with increasing age.

[0036] FIG. 6 depicts the age-dependent distribution of ApoE genotypes (see, Schächter et al. (1994) *Nature Genetics* 6:29-32).

[0037] FIGS. 7A-D depicts age-related and genotype frequency of the p53 (tumor suppressor) codon 72 among the Caucasian population in the database. *R72 and *P72 represent the frequency of the allele in the database population. R72, R72P, and P72 represent the genotypes of the individuals in the population. The frequency of the homozygous P72 allele drops from 6.7% to 3.7% with age.

[0038] FIG. 8 depicts the allele and genotype frequencies of the p21 S31R allele as a function of age.

[0039] FIG. 9 depicts the frequency of the FVII Allele 353Q in pooled versus individual samples.

[0040] FIG. 10 depicts the frequency of the CETP (cholesterol ester transfer protein) allele in pooled versus individual samples.

[0041] FIG. 11 depicts the frequency of the plasminogen activator inhibitor-1 (PAI-1) 5G in pooled versus individual samples.

[0042] FIG. 12 shows mass spectra of the samples and the ethnic diversity of the PAI-1 alleles.

[0043] FIG. 13 shows mass spectra of the samples and the ethnic diversity of the CETP 405 alleles.

[0044] FIG. 14 shows mass spectra of the samples and the ethnic diversity of the Factor VII 353 alleles.

[0045] FIG. 15 shows ethnic diversity of PAI-1, CETP and Factor VII using the pooled DNA samples.

[0046] FIG. 16 shows the p53-Rb pathway and the relationships among the various factors in the pathway.

[0047] FIG. 17, which is a block diagram of a computer constructed to provide and process the databases described herein, depicts a typical computer system for storing and sorting the databases provided herein and practicing the methods provided herein.

[0048] FIG. 18 is a flow diagram that illustrates the processing steps performed using the computer illustrated in FIG. 17, to maintain and provide access to the databases for identifying polymorphic genetic markers.

[0049] FIG. 19 is a histogram showing the allele and genotype distribution in the age and sex stratified Caucasian population for the AKAP10-1 locus. Bright green bars show frequencies in individuals younger than 40 years. Dark green bars show frequencies in individuals older than 60 years.

[0050] FIG. 20 is a histogram showing the allele and genotype distribution in the age and sex stratified Caucasian population for the AKAP10-5 locus. Bright green bars show frequencies in individuals younger than 40 years; dark green bars show frequencies in individuals older than 60 years.

[0051] FIG. 21 is a histogram showing the allele and genotype distribution in the age and sex stratified Caucasian population for the h-msrA locus. Genotype difference between male age groups is significant. Bright green bars show frequencies in individuals younger than 40 years. Dark green bars show frequencies in individuals older than 60 years.

[0052] FIGS. 22A-D is a sample data collection questionnaire used for the healthy database.

[0053] FIG. 23 is a flowchart showing processing performed by the computing device of FIG. 24 when performing genotyping of sense strands and antisense strands from assay fragments.

[0054] FIG. 24 is a block diagram showing a system provided herein;

[0055] FIG. 25 is a flowchart of a method of identifying a biological sample provided herein;

[0056] FIG. 26 is a graphical representation of data from a mass spectrometer;

[0057] FIG. 27 is a diagram of wavelet transformation of mass spectrometry data;

[0058] FIG. 28 is a graphical representation of wavelet stage 0 hi data;

[0059] FIG. 29 is a graphical representation of stage 0 noise profile;

[0060] FIG. 30 is a graphical representation of generating stage noise standard deviations;

[0061] FIG. 31 is a graphical representation of applying a threshold to data stages;

[0062] FIG. 32 is a graphical representation of a sparse data set;

[0063] FIG. 33 is a formula for signal shifting;

[0064] FIG. 34 is a graphical representation of a wavelet transformation of a denoised and shifted signal;

[0065] FIG. 35 is a graphical representation of a denoised and shifted signal;

[0066] FIG. 36 is a graphical representation of removing peak sections;

[0067] FIG. 37 is a graphical representation of generating a peak free signal;

[0068] FIG. 38 is a block diagram of a method of generating a baseline correction;

[0069] FIG. 39 is a graphical representation of a baseline and signal;

[0070] FIG. 40 is a graphical representation of a signal with baseline removed;

[0071] FIG. 41 is a table showing compressed data;

[0072] FIG. 42 is a flowchart of method for compressing data;

[0073] FIG. 43 is a graphical representation of mass shifting;

[0074] FIG. 44 is a graphical representation of determining peak width;

[0075] FIG. 45 is a graphical representation of removing peaks;

[0076] FIG. 46 is a graphical representation of a signal with peaks removed;

[0077] FIG. 47 is a graphical representation of a residual baseline;

[0078] FIG. 48 is a graphical representation of a signal with residual baseline removed;

[0079] FIG. 49 is a graphical representation of determining peak height;

[0080] FIG. 50 is a graphical representation of determining signal-to-noise for each peak;

[0081] FIG. 51 is a graphical representation of determining a residual error for each peak;

[0082] FIG. 52 is a graphical representation of peak probabilities;

[0083] FIG. 53 is a graphical representation of applying an allelic ratio to peak probability;

[0084] FIG. 54 is a graphical representation of determining peak probability;

[0085] FIG. 55 is a graphical representation of calling a genotype;

[0086] FIG. 56 is a flowchart showing a statistical procedure for calling a genotype;

[0087] FIG. 57 is a flowchart showing processing performed by the computing device of FIG. 1 when performing standardless genotyping; and

[0088] FIG. 58 is graphical representation of applying an allelic ratio to peak probability for standardless genotype processing.

DETAILED DESCRIPTION

[0089] Definitions

[0090] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art to which this invention belongs. All patents, applications, published applications and other publications and sequences from GenBank and other databases referred to herein throughout the disclosure are incorporated by reference in their entirety.

[0091] As used herein, a biopolymer includes, but is not limited to, nucleic acid, proteins, polysaccharides, lipids and other macromolecules. Nucleic acids include DNA, RNA, and fragments thereof. Nucleic acids can be derived from genomic DNA, RNA, mitochondrial nucleic acid, chloroplast nucleic acid and other organelles with separate genetic material.

[0092] As used herein, morbidity refers to conditions, such as diseases or disorders, that compromise the health and well-being of an organism, such as an animal. Morbidity susceptibility or morbidity-associated genes are genes that, when altered, for example, by a variation in nucleotide sequence, facilitate the expression of a specific disease clinical phenotype. Thus, morbidity susceptibility genes have the potential, upon alteration, of increasing the likelihood or general risk that an organism will develop a specific disease.

[0093] As used herein, mortality refers to the statistical likelihood that an organism, particularly an animal, will not survive a full predicted lifespan. Hence, a trait or a marker, such as a polymorphism, associated with increased mortality is observed at a lower frequency in older than younger segments of a population.

[0094] As used herein, a polymorphism, e.g. genetic variation, refers to a variation in the sequence of a gene in the genome amongst a population, such as allelic variations and other variations that arise or are observed. Thus, a polymorphism refers to the occurrence of two or more genetically determined alternative sequences or alleles in a population. These differences can occur in coding and non-coding portions of the genome, and can be manifested or detected as differences in nucleic acid sequences, gene expression, including, for example transcription, processing, translation, transport, protein processing, trafficking, DNA synthesis, expressed proteins, other gene products or products of biochemical pathways or in post-translational modifications and any other differences manifested amongst members of a population. A single nucleotide polymorphism (SNP) refers to a polymorphism that arises as the result of a single base change, such as an insertion, deletion or change in a base.

[0095] A polymorphic marker or site is the locus at which divergence occurs. Such site can be as small as one base pair (an SNP). Polymorphic markers include, but are not limited to, restriction fragment length polymorphisms, variable number of tandem repeats (VNTR's), hypervariable regions, minisatellites, dinucleotide repeats, trinucleotide repeats, tetranucleotide repeats and other repeating patterns, simple sequence repeats and insertional elements, such as Alu. Polymorphic forms also are manifested as different mendelian alleles for a gene. Polymorphisms can be observed by differences in proteins, protein modifications, RNA expression modification, DNA and RNA methylation, regulatory

factors that alter gene expression and DNA replication, and any other manifestation of alterations in genomic nucleic acid or organelle nucleic acids.

[0096] As used herein, a healthy population refers to a population of organisms, including but are not limited to, animals, bacteria, viruses, parasites, plants, eubacteria, and others, that are disease free. The concept of disease-free is a function of the selected organism. For example, for mammals it refers to a subject not manifesting any disease state. Practically a healthy subject, when human, is defined as human donor who passes blood bank criteria to donate blood for eventual use in the general population. These criteria are as follows: free of detectable viral, bacterial, mycoplasma, and parasitic infections; not anemic; and then further selected based upon a questionnaire regarding history (see **FIG. 3**). Thus, a healthy population represents an unbiased population of sufficient health to donate blood according to blood bank criteria, and not further selected for any disease state. Typically such individuals are not taking any medications. For plants, for example, it is a plant population that does not manifest diseases pathology associated with plants. For bacteria it is a bacterial population replicating without environmental stress, such as selective agents, heat and other pathogens.

[0097] As used herein, a healthy database (or healthy patient database) refers to a database of profiles of subjects that have not been pre-selected for any particular disease. Hence, the subjects that serve as the source of data for the database are selected, according to predetermined criteria, to be healthy. In contrast to other such databases that have been pre-selected for subjects with a particular disease or other characteristic, the subjects for the database provided herein are not so-selected. Also, if the subjects do manifest a disease or other condition, any polymorphism discovered or characterized should be related to an independent disease or condition. In a one embodiment, where the subjects are human, a healthy subject manifests no disease symptoms and meets criteria, such as those set by blood banks for blood donors.

[0098] Thus, the subjects for the database are a population of any organism, including, but are not limited to, animals, plants, bacteria, viruses, parasites and any other organism or entity that has nucleic acid. Among subjects are mammals, such as, although not necessarily, humans. Such a database can capture the diversity of a population, thus providing for discovery of rare polymorphisms.

[0099] As used herein, a profile refers to information relating to, but not limited to and not necessarily including all of, age, sex, ethnicity, disease history, family history, phenotypic characteristics, such as height and weight and other relevant parameters. A sample collect information form is shown in **FIG. 22**, which illustrates profile intent.

[0100] As used herein, a disease state is a condition or abnormality or disorder that can be inherited or result from environmental stresses, such as toxins, bacterial, fungal and viral infections.

[0101] As used herein, set of non-selected subjects means that the subjects have not been pre-selected to share a common disease or other characteristic. They can be selected to be healthy as defined herein.

[0102] As used herein, a phenotype refers to a set of parameters that includes any distinguishable trait of an

organism. A phenotype can be physical traits and can be, in instances in which the subject is an animal, a mental trait, such as emotional traits. Some phenotypes can be determined by observation elicited by questionnaires (see, e.g., **FIGS. 3 and 22**) or by referring to prior medical and other records. For purposes herein, a phenotype is a parameter around which the database can be sorted.

[0103] As used herein, a parameter is any input data that will serve as a basis for sorting the database. These parameters will include phenotypic traits, medical histories, family histories and any other such information elicited from a subject or observed about the subject. A parameter can describe the subject, some historical or current environmental or social influence experienced by the subject, or a condition or environmental influence on someone related to the subject. Parameters include, but are not limited to, any of those described herein, and known to those of skill in the art.

[0104] As used herein, haplotype refers to two or polymorphism located on a single DNA strand. Hence, haplotyping refers to identification of two or more polymorphisms on a single DNA strand. Haplotypes can be indicative of a phenotype. For some disorders a single polymorphism can suffice to indicate a trait; for others a plurality (i.e., a haplotype) can be needed. Haplotyping can be performed by isolating nucleic acid and separating the strands. In addition, when using enzymes such as certain nucleases, that produce, different size fragments from each strand, strand separation is not needed for haplotyping.

[0105] As used herein, pattern with reference to a mass spectrum or mass spectrometric analyses, refers to a characteristic distribution and number of signals (such peaks or digital representations thereof).

[0106] As used herein, signal in the context of a mass spectrum and analysis thereof refers to the output data, which the number or relative number of molecules having a particular mass. Signals include "peaks" and digital representations thereof.

[0107] As used herein, adaptor, when used with reference to haplotyping using Fen ligase, refers to a nucleic acid that specifically hybridizes to a polymorphism of interest. An adaptor can be partially double-stranded. An adaptor complex is formed when an adaptor hybridizes to its target.

[0108] As used herein, a target nucleic acid refers to any nucleic acid of interest in a sample. It can contain one or more nucleotides.

[0109] As used herein, standardless analysis refers to a determination based upon an internal standard. For example, the frequency of a polymorphism can be determined herein by comparing signals within a single mass spectrum.

[0110] As used herein, amplifying refers to methods for increasing the amount of a bipolymer, especially nucleic acids. Based on the 5' and 3' primers that are chosen, amplification also serves to restrict and define the region of the genome which is subject to analysis. Amplification can be performed by any method known to those skilled in the art, including use of the polymerase chain reaction (PCR) etc. Amplification, e.g., PCR must be done quantitatively when the frequency of polymorphism is required to be determined.

[0111] As used herein, cleaving refers to non-specific and specific fragmentation of a biopolymer.

[0112] As used herein, multiplexing refers to the simultaneous detection of more than one polymorphism. Methods for performing multiplexed reactions, particularly in conjunction with mass spectrometry are known (see, e.g., U.S. Pat. Nos. 6,043,031, 5,547,835 and International PCT application No. WO 97/37041).

[0113] As used herein, reference to mass spectrometry encompasses any suitable mass spectrometric format known to those of skill in the art. Such formats include, but are not limited to, Matrix-Assisted Laser Desorption/Ionization, Time-of-Flight (MALDI-TOF), Electrospray (ES), IR-MALDI (see, e.g., published International PCT application No. 99/57318 and U.S. Pat. No. 5,118,937), Ion Cyclotron Resonance (ICR), Fourier Transform and combinations thereof. MALDI, particular UV and IR, are among the formats contemplated.

[0114] As used herein, mass spectrum refers to the presentation of data obtained from analyzing a biopolymer or fragment thereof by mass spectrometry either graphically or encoded numerically.

[0115] As used herein, a blood component is a component that is separated from blood and includes, but is not limited to red blood cells and platelets, blood clotting factors, plasma, enzymes, plasminogen, immunoglobulins. A cellular blood component is a component of blood, such as a red blood cell, that is a cell. A blood protein is a protein that is normally found in blood. Examples of such proteins are blood factors VII and VIII. Such proteins and components are well-known to those of skill in the art.

[0116] As used herein, plasma can be prepared by any method known to those of skill in the art. For example, it can be prepared by centrifuging blood at a force that pellets the red cells and forms an interface between the red cells and the buffy coat, which contains leukocytes, above which is the plasma. For example, typical platelet concentrates contain at least about 10% plasma.

[0117] Blood can be separated into its components, including, but not limited to, plasma, platelets and red blood cells by any method known to those of skill in the art. For example, blood can be centrifuged for a sufficient time and at a sufficient acceleration to form a pellet containing the red blood cells. Leukocytes collect primarily at the interface of the pellet and supernatant in the buffy coat region. The supernatant, which contains plasma, platelets, and other blood components, can then be removed and centrifuged at a higher acceleration, whereby the platelets pellet.

[0118] As used herein, p53 is a cell cycle control protein that assesses DNA damage and acts as a transcription factor regulation gene which control cell growth, DNA repair and apoptosis. The p53 mutations have been found in a wide variety of different cancers, including all of the different types of leukemia, with varying frequency. The loss of normal p53 functions results in genomic instability and uncontrolled growth of the host cell.

[0119] As used herein, p21 is a cyclin-dependent kinase inhibitor, associated with G1 phase arrest of normal cells. Expression triggers apoptosis or programmed cell death and has been associated with Wilms' tumor, a pediatric kidney cancer.

[0120] As used herein, Factor VII is a serine protease involved the extrinsic blood coagulation cascade. This factor is activated by thrombin and works with tissue factor (Factor III) in the processing of Factor X to Factor Xa. Evidence has supported an association between polymorphisms in the gene and increase Factor VII activity which can result in an elevated risk of ischemic cardiovascular disease including myocardial infarction.

[0121] As used herein, a relational database stores information in a form representative of matrices, such as two-dimensional tables, including rows and columns of data, or higher dimensional matrices. For example, in one embodiment, the relational database has separate tables each with a parameter. The tables are linked with a record number, which also acts as an index. The database can be searched or sorted by using data in the tables and is stored in any suitable storage medium, such as floppy disk, CD rom disk, hard drive or other suitable medium.

[0122] As used herein, a bar codes refers any array of optically readable marks of any desired size and shape that are arranged in a reference context or frame of, typically, although not necessarily, one or more columns and one or more rows. For purposes herein, the bar code refers to any symbology, not necessary "bar" but can include dots, characters or any symbol or symbols.

[0123] As used herein, symbology refers to an identifier code or symbol, such as a bar code, that is linked to a sample. The index will reference each such symbology. The symbology is any code known or designed by the user. The symbols are associated with information stored in the database. For example, each sample can be uniquely identified with an encoded symbology. The parameters, such as the answers to the questions and subsequent genotypic and other information obtained upon analysis of the samples is included in the database and associated with the symbology. The database is stored on any suitable recording medium, such as a hard drive, a floppy disk, a tape, a CD ROM, a DVD disk and any other suitable medium.

[0124] DATABASES

[0125] Human genotyping is currently dependent on collaborations with hospitals, tissues banks and research institutions that provide samples of disease tissue. This approach is based on the concept that the onset and/or progression of diseases can be correlated with the presence of a polymorphisms or other genetic markers. This approach does not consider that disease correlated with the presence of specific markers and the absence of specific markers. It is shown herein that identification and scoring of the appearance and disappearance of markers is possible only if these markers are measured in the background of healthy subjects where the onset of disease does not mask the change in polymorphism occurrence. Databases of information from disease populations suffer from small sample size, selection bias and heterogeneity. The databases provided herein from healthy populations solve these problems by permitting large sample bands, simple selection methods and diluted heterogeneity.

[0126] Provided herein are first databases of parameters, associated with non-selected, particularly healthy, subjects. Also provided are combinations of the databases with indexed samples obtained from each of the subjects. Further provided are databases produced from the first databases.

These contain, in addition to the original parameters, information, such as genotypic information, including, but are not limited to, genomic sequence information, derived from the samples.

[0127] The databases, which are herein designated healthy databases, are so-designated because they are not obtained from subjects pre-selected for a particular disease. Hence, although individual members can have a disease, the collection of individuals is not selected to have a particular disease.

[0128] The subjects from whom the parameters are obtained comprise either a set of subjects who are randomly selected across, typically, all populations, or are pre-selected to be disease-free or healthy. As a result, the database is not selected to be representative of any pre-selected phenotype, genotype, disease or other characteristic. Typically the number of subjects from which the database is prepared is selected to produce statistically significant results when used in the methods provided herein. Generally, the number of subjects will be greater than 100, 200, and typically than 1000. The precise number can be empirically determined based upon the frequency of the parameter(s) that can be used to sort the database. Generally the population can have at least 50, at least 100, at least 200, at least 500, at least 1000, at least 5000 or at least 10,000 or more subjects.

[0129] Upon identification of a collection of subjects, information about each subject is recorded and associated with each subject as a database. The information associated with each of the subjects, includes, but is not limited to, information related to historical characteristics of the subjects, phenotypic characteristics and also genotypic characteristics, medical characteristics and any other traits and characteristics about the subject that can be determined. This information will serve as the basis for sorting the database.

[0130] In an exemplary embodiment, the subjects are mammals, such as humans, and the information relates to one or more of parameters, such as age, sex, medical history, ethnicity and any other factor. Such information, when the animals are humans, for example, can be obtained by a questionnaire and by observations about the individual, such as hair color, eye color and other characteristics. Genotypic information can be obtained from tissue or other body and body fluid samples from the subject.

[0131] The healthy genomic database can include profiles and polymorphisms from healthy individuals from a library of blood samples where each sample in the library is an individual and separate blood or other tissue sample. Each sample in the database is profiled as to the sex, age, ethnic group, and disease history of the donor.

[0132] The databases are generated by first identifying healthy populations of subjects and obtaining information about each subject that will serve as the sorting parameters for the database. This information can be entered into a storage medium, such as the memory of a computer.

[0133] The information obtained about each subject in a population used for generating the database is stored in a computer memory or other suitable storage medium. The information is linked to an identifier associated with each subject. Hence the database will identify a subject, for example by a datapoint representative of a bar code, and then all information, such as the information from a ques-

tionnaire, regarding the individual is associated with the datapoint. As the information is collected the database is generated.

[0134] Thus, for example, profile information, such as subject histories obtained from questionnaires, is collected in the database. The resulting database can be sorted as desired, using standard software, such as by age, sex and/or ethnicity. An exemplary questionnaire for subjects from whom samples are to be obtained is shown in FIGS. 22A-D. Each questionnaire, for example, can be identified by a bar code, particularly a machine readable bar code for entry into the database. After a subject provides data and is deemed to be healthy (i.e., meets standards for blood donation), the data in the questionnaire is entered into the database and is associated with the bar code. A tissue, cell or blood sample is obtained from the subject.

[0135] FIG. 4 exemplifies processing and tracking of blood sample components. Each component is tracked with a bar code, dated, is entered into the database and associated with the subject and the profile of the subject. Typically, the whole blood is centrifuged to produce plasma, red blood cells (which pellet) and leukocytes found in the buffy coat which layers in between. Various samples are obtained and coded with a bar code and stored for use as needed.

[0136] Samples are collected from the subjects. The samples include, but are not limited to, tissues, cells, and fluids, such as nucleic acid, blood, plasma, amniotic fluid, synovial fluid, urine, saliva, aqueous humor, sweat, sperm samples and cerebral spinal fluid. It is understood that the particular set of samples depends upon the organisms in the population.

[0137] Once samples are obtained the collection can be stored and, in some embodiments, each sample is indexed with an identifier, particularly a machine readable code, such as a bar code. For analyses, the samples or components of the samples, particularly biopolymers and small molecules, such as nucleic acids and/or proteins and metabolites, are isolated.

[0138] After samples are analyzed, this information is entered into the database in the memory of the storage medium and associated with each subject. This information includes, but is not limited to, genotypic information. Particularly, nucleic acid sequence information and other information indicative of polymorphisms, such as masses of PCR fragments, peptide fragment sequences or masses, spectra of biopolymers and small molecules and other indicia of the structure or function of a gene, gene product or other marker from which the existence of a polymorphism within the population can be inferred.

[0139] In an exemplary embodiment, a database can be derived from a collection of blood samples. For example, FIG. 1 (see, also FIG. 10) shows the status of a collection of over 5000 individual samples. The samples were processed in the laboratory following SOP (standard operating procedure) guidelines. Any standard blood processing protocol can be used.

[0140] For the exemplary database described herein, the following criteria were used to select subjects:

[0141] No testing is done for infectious agents.

[0142] Age: At least 17 years old

[0143] Weight: Minimum of 110 pounds

[0144] Permanently Disqualified:

- [0145]** History of hepatitis (after age 11)
- [0146]** Leukemia Lymphoma
- [0147]** Human immunodeficiency virus (HIV), AIDS
- [0148]** Chronic kidney disease

[0149] Temporarily Disqualified:

- [0150]** Pregnancy—until six weeks after delivery, miscarriage or abortion
- [0151]** Major surgery or transfusions—for one year
- [0152]** Mononucleosis—until complete recovery
- [0153]** Prior whole blood donation—for eight weeks
- [0154]** Antibiotics by injection for one week; by mouth, for forty-eight hours, except antibiotics for skin complexion;

[0155] 5 year Deferment:

- [0156]** Internal cancer and skin cancer if it has been removed, is healed and there is no recurrence

[0157] These correspond to blood bank criteria for donating blood and represent a healthy population as defined herein for a human healthy database.

[0158] Structure of the Database

[0159] Any suitable database structure and format known to those of skill in the art can be employed. For example, a relational database is an exemplary format in which data are stored as matrices or tables of the parameters linked by an indexer that identifies each subject. Software for preparing and manipulating, including sorting the database, can be readily developed or adapted from commercially available software, such as Microsoft Access.

[0160] Quality Control

[0161] Quality control procedures can be implemented. For example, after collection of samples, the quality of the collection in the bank can be assessed. For example, mix-up of samples can be checked by testing for known markers, such as sex. After samples are separated by ethnicity, samples are randomly tested for a marker associated with a particular ethnicity, such as HLA DQA1 group specific component, to assess whether the samples have been properly sorted by ethnic group. An exemplary sample bank is depicted in FIG. 4.

[0162] Obtaining Genotypic Data and Other Parameters for the Database

[0163] After informational and historical parameters are entered into the database, material from samples obtained from each subject, is analyzed. Analyzed material include proteins, metabolites, nucleic acids, lipids and any other desired constituent of the material. For example, nucleic acids, such as genomic DNA, can be analyzed by sequencing.

[0164] Sequencing can be performed using any method known to those of skill in the art. For example, if a polymorphism is identified or known, and it is desired to assess its frequency or presence among the subjects in the database, the region of interest from each sample can be

isolated, such as by PCR or restriction fragments, hybridization or other suitable method known to those of skill in the art and sequenced. For purposes herein, sequencing analysis can be effected using mass spectrometry (see, e.g., U.S. Pat. Nos. 5,547,835, 5,622,824, 5,851,765, and 5,928,906). Nucleic acids also can be sequenced by hybridization (see, e.g., U.S. Pat. Nos. 5,503,980, 5,631,134, 5,795,714) and including analysis by mass spectrometry (see, U.S. application Ser. Nos. 08/419,994 and 09/395,409).

[0165] In other detection methods, it is necessary to first amplify prior to identifying the allelic variant. Amplification can be performed, e.g., by PCR and/or LCR, according to methods known in the art. In one embodiment, genomic DNA of a cell is exposed to two PCR primers and amplification for a number of cycles sufficient to produce the required amount of amplified DNA. In some embodiments, the primers are located between 150 and 350 base pairs apart.

[0166] Alternative amplification methods include: self sustained sequence replication (Guatelli, J. C. et al., 1990, Proc. Natl. Acad. Sci. U.S.A. 87:1874-1878), transcriptional amplification system (Kwoh, D. Y. et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86:1173-1177), Q-Beta Replicase (Lizardi, P. M. et al., 1988, Bio/Technology 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

[0167] Nucleic acids also can be analyzed by detection methods and protocols, particularly those that rely on mass spectrometry (see, e.g., U.S. Pat. No. 5,605,798, 6,043,031, allowed copending U.S. application Ser. No. 08/744,481, U.S. application Ser. No. 08/990,851 and International PCT application No. WO 99/31278, International PCT application No. WO 98/2001 9). These methods can be automated (see, e.g., copending U.S. application Ser. No. 09/285,481 and published International PCT application No. PCT/US00/08111, which describes an automated process line). Among the methods of analysis herein are those involving the primer oligo base extension (PROBE) reaction with mass spectrometry for detection (described herein and elsewhere, see e.g., U.S. Pat. No. 6,043,031; see, also U.S. application Ser. Nos. 09/287,681, 09/287,682, 09/287,141 and 09/287,679, allowed copending U.S. application Ser. No. 08/744,481, International PCT application No. PCT/US97/20444, published as International PCT application No. WO 98/20019, and based upon U.S. application Ser. Nos. 08/744,481, 08/744,590, 08/746,036, 08/746,055, 08/786,988, 08/787,639, 08/933,792, 08/746,055, 08/786,988 and 08/787,639; see, also U.S. application Ser. No. 09/074,936, U.S. Pat. No. 6,024,925, and U.S. application Ser. Nos. 08/746,055 and 08/786,988, and published International PCT application No. WO 98/20020)

[0168] A chip based format in which the biopolymer is linked to a solid support, such as a silicon or silicon-coated substrate, such as in the form of an array, is among the formats for performing the analyses is. Generally, when analyses are performed using mass spectrometry, particularly MALDI, small nanoliter volumes of sample are loaded on, such that the resulting spot is about, or smaller than, the

size of the laser spot. It has been found that when this is achieved, the results from the mass spectrometric analysis are quantitative. The area under the signals in the resulting mass spectra are proportional to concentration (when normalized and corrected for background). Methods for preparing and using such chips are described in U.S. Pat. No. 6,024,925, co-pending U.S. application Ser. Nos. 08/786,988, 09/364,774, 09/371,150 and 09/297,575; see, also U.S. application Ser. No. PCT/US97/20195, which published as WO 98/20020. Chips and kits for performing these analyses are commercially available from SEQUENOM under the trademark MassARRAY. MassArray relies on the fidelity of the enzymatic primer extension reactions combined with the miniaturized array and MALDI-TOF (Matrix-Assisted Laser Desorption Ionization-Time of Flight) mass spectrometry to deliver results rapidly. It accurately distinguishes single base changes in the size of DNA fragments associated with genetic variants without tags.

[0169] The methods provided herein permit quantitative determination of alleles. The areas under the signals in the mass spectra can be used for quantitative determinations. The frequency is determined from the ratio of the signal to the total area of all of the spectrum and corrected for background. This is possible because of the PROBE technology as described in the above applications incorporated by reference herein.

[0170] Additional methods of analyzing nucleic acids include amplification-based methods including polymerase chain reaction (PCR), ligase chain reaction (LCR), mini-PCR, rolling circle amplification, autocatalytic methods, such as those using Q β replicase, TAS, 3SR, and any other suitable method known to those of skill in the art.

[0171] Other methods for analysis and identification and detection of polymorphisms, include but are not limited to, allele specific probes, Southern analyses, and other such analyses.

[0172] The methods described below provide ways to fragment given amplified or non-amplified nucleotide sequences thereby producing a set of mass signals when mass spectrometry is used to analyze the fragment mixtures.

[0173] Amplified fragments are yielded by standard polymerase chain methods (U.S. Pat. Nos. 4,683,195 and 4,683,202). The fragmentation method involves the use of enzymes that cleave single or double strands of DNA and enzymes that ligate DNA. The cleavage enzymes can be glycosylases, nickases, and site-specific and non site-specific nucleases, such as, but are not limited to, glycosylases, nickases and site-specific nucleases.

[0174] Glycosylase Fragmentation Method

[0175] DNA glycosylases specifically remove a certain type of nucleobase from a given DNA fragment. These enzymes can thereby produce abasic sites, which can be recognized either by another cleavage enzyme, cleaving the exposed phosphate backbone specifically at the abasic site and producing a set of nucleobase specific fragments indicative of the sequence, or by chemical means, such as alkaline solutions and or heat. The use of one combination of a DNA glycosylase and its targeted nucleotide would be sufficient to generate a base specific signature pattern of any given target region.

[0176] Numerous DNA glycosylases are known. For example, a DNA glycosylase can be uracil-DNA glycosylase (UDG), 3-methyladenine DNA glycosylase, 3-methyladenine DNA glycosylase II, pyrimidine hydrate-DNA glycosylase, FaPy-DNA glycosylase, thymine mismatch-DNA glycosylase, hypoxanthine-DNA glycosylase, 5-Hydroxymethyluracil DNA glycosylase (HmUDG), 5-Hydroxymethylcytosine DNA glycosylase, or 1,N6-etheno-adenine DNA glycosylase (see, e.g., U.S. Pat. Nos. 5,536,649, 5,888,795, 5,952,176 and 6,099,553, International PCT application Nos. WO 97/03210, WO 99/54501; see, also, Eftedal et al. (1993) *Nucleic Acids Res* 21:2095-2101, Bjelland and Seeberg (1987) *Nucleic Acids Res.* 15:2787-2801, Saparbaev et al. (1995) *Nucleic Acids Res.* 23:3750-3755, Bessho (1999) *Nucleic Acids Res.* 27:979-983) corresponding to the enzyme's modified nucleotide or nucleotide analog target. uracil-DNA glycosylase (UDG) is an exemplary glycosylase.

[0177] Uracil, for example, can be incorporated into an amplified DNA molecule by amplifying the DNA in the presence of normal DNA precursor nucleotides (e.g. dCTP, dATP, and dGTP) and dUTP. When the amplified product is treated with UDG, uracil residues are cleaved. Subsequent chemical treatment of the products from the UDG reaction results in the cleavage of the phosphate backbone and the generation of nucleobase specific fragments. Moreover, the separation of the complementary strands of the amplified product prior to glycosylase treatment allows complementary patterns of fragmentation to be generated. Thus, the use of dUTP and Uracil DNA glycosylase allows the generation of T specific fragments for the complementary strands, thus providing information on the T as well as the A positions within a given sequence. Similar to this, a C-specific reaction on both (complementary) strands (i.e. with a C-specific glycosylase) yields information on C as well as G positions within a given sequence if the fragmentation patterns of both amplification strands are analyzed separately. Thus, with the glycosylase method and mass spectrometry, a full series of A, C, G and T specific fragmentation patterns can be analyzed.

[0178] Nickase Fragmentation Method

[0179] A DNA nickase, or DNase, can be used to recognize and cleave one strand of a DNA duplex. Numerous nickases are known. Among these, for example, are nickase NY2A nickase and NYS1 nickase (Megabase) with the following cleavage sites:

[0180] NY2A:

[0181] 5' . . . R AG . . . 3'

[0182] 3' . . . YTC . . . 5' where R=A or G and Y=C or T

[0183] NYS1:

[0184] 5' . . . CC[A/G/T] . . . 3'

[0185] 3' . . . GG[T/C/A] . . . 5'.

[0186] Fen-Ligase Fragmentation Method

[0187] The Fen-ligase method involves two enzymes: Fen-1 enzyme and a ligase. The Fen-1 enzyme is a site-specific nuclease known as a "flap" endonuclease (U.S. Pat. Nos. 5,843,669, 5,874,283, and 6,090,606). This enzyme recognizes and cleaves DNA "flaps" created by the overlap

of two oligonucleotides hybridized to a target DNA strand. This cleavage is highly specific and can recognize single base pair mutations, permitting detection of a single homologue from an individual heterozygous at one SNP of interest and then genotyping that homologue at other SNPs occurring within the fragment. Fen-1 enzymes can be Fen-1 like nucleases e.g. human, murine, and *Xenopus* XPG enzymes and yeast RAD2 nucleases or Fen-1 endonucleases from, for example, *M. jannaschii*, *P. furiosus*, and *P. woesei*. Among such enzymes are the Fen-1 enzymes.

[0188] The ligase enzyme forms a phosphodiester bond between two double stranded nucleic acid fragments. The ligase can be DNA Ligase I or DNA Ligase III (see, e.g., U.S. Pat. Nos. U.S. 5,506,137, 5,700,672, 5,858,705 and 5,976,806; see, also, Waga, et al. (1994) J. Biol. Chem. 269:10923-10934, Li et al. (1994) Nucleic Acids Res. 22:632-638, Arrand et al. (1986) J. Biol. Chem. 261:9079-9082, Lehman (1974) Science 186:790-797, Higgins and Cozzarelli (1979) Methods Enzymol. 68:50-71, Lasko et al. (1990) Mutation Res. 236:277-287, and Lindahl and Barnes (1992) Ann. Rev. Biochem. 61:251-281). Thermostable ligase (Epicenter Technologies), where "thermostable" denotes that the ligase retains activity even after exposure to temperatures necessary to separate two strands of DNA, are among the ligases for use herein.

[0189] Type IIS Enzyme Fragmentation Method

[0190] Restriction enzymes bind specifically to and cleave double-stranded DNA at specific sites within or adjacent to a particular recognition sequence. These enzymes have been classified into three groups (e.g. Types I, II, and III) as known to those of skill in the art. Because of the properties of type I and type III enzymes, they have not been widely used in molecular biological applications. Thus, for purposes herein type II enzymes are among those contemplated. Of the thousands of restriction enzymes known in the art, there are 179 different type II specificities. Of the 179 unique type II restriction endonucleases, 31 have a 4-base recognition sequence, 11 have a 5-base recognition sequence, 127 have a 6-base recognition sequence, and 10 have recognition sequences of greater than six bases (U.S. Pat. No. 5,604,098). Of category type II enzymes, type IIS is exemplified herein.

[0191] Type IIS enzymes can be Alw XI, Bbv I, Bce 83, Bpm I, Bsg I, Bsm AI, Bsm FI, Bsa I, Bcc I, Bcg I, Ear I, Eco 57I, Esp 3I, Fau I, Fok I, Gsu I, Hga I, Mme I, Mbo II, Sap I, and the others.

[0192] The Fok I enzyme endonuclease is an exemplary well characterized member of the Type IIS class (see, e.g., U.S. Pat. Nos. 5,714,330, 5,604,098, 5,436,150, 6,054,276 and 5,871,911; see, also, Szybalski et al. (1991) Gene 100:13-26, Wilson and Murray (1991) Ann. Rev. Genet. 25:585-627, Sugisaki et al. (1981) Gene 16:73-78, Podhajska and Szalski (1985) Gene 40:175-182. Fok I recognizes the sequence 5'GGATG-3' and cleaves DNA accordingly. Type IIS restriction sites can be introduced into DNA targets by incorporating the sites into primers used to amplify such targets. Fragments produced by digestion with Fok I are site specific and can be analyzed by mass spectrometry methods such as MALDI-TOF mass spectrometry, ESI-TOF mass spectrometry, and any other type of mass spectrometry well known to those of skill in the art.

[0193] Once a polymorphism has been found to correlate with a parameter such as age, age groups can be screened for

polymorphisms. The possibility of false results due to allelic dropout is examined by doing comparative PCR in an adjacent region of the genome.

[0194] Analyses

[0195] In using the database, allelic frequencies can be determined across the population by analyzing each sample in the population individually, determining the presence or absence of allele or marker of interest in each individual sample, and then determining the frequency of the marker in the population. The database can then be sorted (stratified) to identify any correlations between the allele and a selected parameter using standard statistical analysis. If a correlation is observed, such as a decrease in a particular marker with age or correlation with sex or other parameter, then the marker is a candidate for further study, such as genetic mapping to identify a gene or pathway in which it is involved. The marker can then be correlated, for example, with a disease. Haplotyping also can be carried out. Genetic mapping can be effected using standard methods and can also require use of databases of others, such as databases previously determined to be associated with a disorder.

[0196] Exemplary analyses have been performed and these are shown in the figures, and discussed herein.

[0197] Sample Pooling

[0198] It has been found that using the databases provided herein, or any other database of such information, substantially the same frequencies that were obtained by examining each sample separately can be obtained by pooling samples, such as in batches of 10, 20, 50, 100, 200, 500, 1000 or any other number. A precise number can be determined empirically if necessary, and can be as low as 3.

[0199] In one embodiment, the frequency of genotypic and other markers can be obtained by pooling samples. To do this a target population and a genetic variation to be assessed is selected, a plurality of samples of biopolymers are obtained from members of the population, and the biopolymer from which the marker or genotype can be inferred is determined or detected. A comparison of samples tested in pools and individually and the sorted results therefrom are shown in FIG. 9, which shows frequency of the factor VII Allele 353Q. FIG. 10 depicts the frequency of the CETP Allele in pooled versus individual samples. FIG. 15 shows ethnic diversity among various ethnic groups in the database using pooled DNA samples to obtain the data. FIGS. 12-14 show mass spectra for these samples.

[0200] Pooling of test samples has application not only to the healthy databases provided herein, but also to use in gathering data for entry into any database of subjects and genotypic information, including typical databases derived from diseased populations. What is demonstrated herein, is the finding that the results achieved are statistically the same as the results that would be achieved if each sample is analyzed separately. Analysis of pooled samples by a method, such as the mass spectrometric methods provided herein, permits resolution of such data and quantitation of the results.

[0201] For factor VII the R53Q acid polymorphism was assessed. In FIG. 9, the "individual" data represent allelic frequency observed in 92 individuals reactions. The pooled data represent the allelic frequency of the same 92 individu-

als pooled into a single probe reaction. The concentration of DNA in the samples of individual donors is 250 nanograms. The total concentration of DNA in the pooled samples is also 250 nanograms, where the concentration of any individual DNA is 2.7 nanograms.

[0202] It also was shown that it is possible to reduce the DNA concentration of individuals in a pooled samples from 2.7 nanograms to 0.27 nanograms without any change in the quality of the spectrum or the ability to quantitate the amount of sample detected. Hence low concentrations of sample can be used in the pooling methods.

[0203] Use of the Databases and Markers Identified Thereby

[0204] The successful use of genomics requires a scientific hypothesis (i.e., common genetic variation, such as a SNP), a study design (i.e., complex disorders), samples and technology, such as the chip-based mass spectrometric analyses (see, e.g., U.S. Pat. No. 5,605,798, U.S. Pat. No. 5,777,324, U.S. Pat. No. 6,043,031, allowed copending U.S. application Ser. No. 08/744,481, U.S. application Ser. No. 08/990,851, International PCT application No. WO 98/20019, copending U.S. application Ser. No. 09/285,481, which describes an automated process line for analyses; see, also, U.S. application Ser. Nos. 08/617,256, 09/287,681, 09/287,682, 09/287,141 and 09/287,679, allowed copending U.S. application Ser. No. 08/744,481, International PCT application No. PCT/US97/20444, published as International PCT application No. WO 98/20019, and based upon U.S. application Ser. Nos. 08/744,481, 08/744,590, 08/746,036, 08/746,055, 08/786,988, 08/787,639, 08/933,792, 08/746,055, 09/266,409, 08/786,988 and 08/787,639; see, also U.S. application Ser. No. 09/074,936). All of these aspects can be used in conjunction with the databases provided herein and samples in the collection.

[0205] The databases and markers identified thereby can be used, for example, for identification of previously unidentified or unknown genetic markers and to identify new uses for known markers. As markers are identified, these can be entered into the database to use as sorting parameters from which additional correlations can be determined.

[0206] Previously Unidentified or Unknown Genetic Markers

[0207] The samples in the healthy databases can be used to identify new polymorphisms and genetic markers, using any mapping, sequencing, amplification and other methodologies, and in looking for polymorphisms among the population in the database. The thus-identified polymorphism can then be entered into the database for each sample, and the database sorted (stratified) using that polymorphism as a sorting parameter to identify any patterns and correlations that emerge, such as age correlated changes in the frequency of the identified marker. If a correlation is identified, the locus of the marker can be mapped and its function or effect assessed or deduced.

[0208] Thus, the databases here provide means for:

[0209] identification of significantly different allelic frequencies of genetic factors by comparing the occurrence or disappearance of the markers with increasing age in population and then associating the markers with a disease or a biochemical pathway;

[0210] identification of significantly different allelic frequencies of disease causing genetic factors by comparing the male with the female population or comparing other selected stratified populations and associating the markers with a disease or a biochemical pathway;

[0211] identification of significantly different allelic frequencies of disease causing genetic factors by comparing different ethnic groups and associating the markers with a disease or a biochemical pathway that is known to occur in high frequency in the ethnic group;

[0212] profiling potentially functional variants of genes through the general panmixed population stratified according to age, sex, and ethnic origin and thereby demonstrating the contribution of the variant genes to the physical condition of the investigated population;

[0213] identification of functionally relevant gene variants by gene disequilibrium analysis performed within the general panmixed population stratified according to age, sex, and ethnic origin and thereby demonstrating their contribution to the physical condition of investigated population;

[0214] identification of potentially functional variants of chromosomes or parts of chromosomes by linkage disequilibrium analysis performed within the general panmixed population stratified according to age, sex, and ethnic origin and thereby demonstrating their contribution to the physical condition of investigated population.

[0215] Uses of the Identified Markers and Known Markers

[0216] The databases can also be used in conjunction with known markers and sorted to identify any correlations. For example, the databases can be used for:

[0217] determination and evaluation of the penetrance of medically relevant polymorphic markers;

[0218] determination and evaluation of the diagnostic specificity of medically relevant genetic factors;

[0219] determination and evaluation of the positive predictive value of medically relevant genetic factors;

[0220] determination and evaluation of the onset of complex diseases, such as, but are not limited to, diabetes, hypertension, autoimmune diseases, arteriosclerosis, cancer and other diseases within the general population with respect to their causative genetic factors;

[0221] delineation of the appropriate strategies for preventive disease treatment;

[0222] delineation of appropriate timelines for primary disease intervention;

[0223] validation of medically relevant genetic factors identified in isolated populations regarding their general applicability;

[0224] validation of disease pathways including all potential target structures identified in isolated populations regarding their general applicability; and

[0225] validation of appropriate drug targets identified in isolated populations regarding their general applicability.

[0226] Among the diseases and disorders for which polymorphisms can be linked include, those linked to inborn errors of metabolism, acquired metabolic disorders, intermediary metabolism, oncogenesis pathways, blood clotting pathways, and DNA synthetic and repair pathways, DNA repair/replication/transcription factors and activities, e.g., such as genes related to oncogenesis, aging and genes involved in blood clotting and the related biochemical pathways that are related to thrombosis, embolism, stroke, myocardial infarction, angiogenesis and oncogenesis.

[0227] For example, a number of diseases are caused by or involve deficient or defective enzymes in intermediary metabolism (see, e.g., Tables 1 and 2, below) that result, upon ingestion of the enzyme substrates, in accumulation of harmful metabolites that damage organs and tissues, particularly an infant's developing brain and other organs, resulting in mental retardation and other developmental disorders.

[0228] Identification of Markers and Genes for such Disorders is of Great Interest.

[0229] Model Systems

[0230] Several gene systems, p21, p53 and Lipoprotein Lipase polymorphism (N291S), were selected. The p53 gene is a tumor suppressor gene that is mutated in diverse tumor types. One common allelic variant occurs at codon 72. A polymorphism that has been identified in the p53 gene, i.e., the R72P allele, results in an amino acid exchange, arginine to proline, at codon 72 of the gene.

[0231] Using diseased populations, it has been shown that there are ethnic differences in the allelic distribution of these alleles among African-Americans and Caucasians in the U.S. The results here support this finding and also demonstrate that the results obtained with a healthy database are meaningful (see, FIG. 7B).

[0232] The 291S allele leads to reduced levels of high density lipoprotein cholesterol (HDL-C) that is associated with an increased risk of males for arteriosclerosis and in particular myocardial infarction (see, Reymer et al. (1995) *Nature Genetics* 10:28-34).

[0233] Both genetic polymorphisms were profiled within a part of the Caucasian population-based sample bank. For the polymorphism located in the lipoprotein lipase gene a total of 1025 unselected individuals (436 males and 589 females) were tested. Genomic DNA was isolated from blood samples obtained from the individuals.

[0234] As shown in the Examples and figures, an exemplary database containing about 5000 subjects, answers to the questionnaire (see FIG. 3), and genotypic information has been stratified. A particular known allele has been selected, and the samples tested for the marker using mass spectrometric analyses, particularly PROBE (see the EXAMPLES) to identify polymorphisms in each sample. The population in the database has been sorted according to various parameters and correlations have been observed. For example, FIGS. 2A-C, show sorting of the data by age and sex for the Lipoprotein Lipase gene in the Caucasian population in the database. The results show a decrease in the

frequency of the allele with age in males but no such decrease in females. Other alleles that have been tested against the database, include, alleles of p53, p21 and factor VII. Results when sorted by age are shown in the figures.

[0235] These examples demonstrate an effect of altered frequency of disease causing genetic factors within the general population. The scientific interpretation of those results allows prediction of medical relevance of polymorphic genetic alterations. In addition, conclusions can be drawn with regard to their penetrance, diagnostic specificity, positive predictive value, onset of disease, most appropriate onset of preventive strategies, and the general applicability of genetic alterations identified in isolated populations to panmixed populations.

[0236] Therefore, an age- and sex-stratified population-based sample bank that is ethnically homogenous is a suitable tool for rapid identification and validation of genetic factors regarding their potential medical utility.

[0237] Exemplary Computer System for Creating, Storing and Processing the Databases

[0238] Systems

[0239] Systems, including computers, containing the databases are provided herein. The computers and databases can be used in conjunction, for example, with the APL system (see, copending U.S. application Ser. No. 09/285,481), which is an automated system for analyzing biopolymers, particularly nucleic acids. Results from the APL system can be entered into the database.

[0240] Any suitable computer system can be used. The computer system can be integrated into systems for sample analysis, such as the automated process line described herein (see, e.g., copending U.S. application Ser. No. 09/285,481).

[0241] FIG. 17 is a block diagram of a computer constructed to provide and process the databases described herein. The processing that maintains the database and performs the methods and procedures can be performed on multiple computers all having a similar construction, or can be performed by a single, integrated computer. For example, the computer through which data are added to the database can be separate from the computer through which the database is sorted, or can be integrated with it. In either arrangement, the computers performing the processing can have a construction as illustrated in FIG. 17.

[0242] FIG. 17 is a block diagram of an exemplary computer 1700 that maintains the database described above and performs the methods and procedures. Each computer 1700 operates under control of a central processor unit (CPU) 1702, such as a "Pentium" microprocessor and associated integrated circuit chips, available from Intel Corporation of Santa Clara, Calif., USA. A computer user can input commands and data from a keyboard and display mouse 1704 and can view inputs and computer output at a display 1706. The display is typically a video monitor or flat panel display device. The computer 1700 also includes a direct access storage device (DASD) 1707, such as a fixed hard disk drive. The memory 1708 typically comprises volatile semiconductor random access memory (RAM). Each computer can include a program product reader 1710 that accepts a program product storage device 1712, from which the program product reader can read data (and to

which it can optionally write data). The program product reader can comprise, for example, a disk drive, and the program product storage device can comprise removable storage media such as a magnetic floppy disk, an optical CD-ROM disc, a CD-R disc, a CD-RW disc, or a DVD data disc. If desired, the computers can be connected so they can communicate with each other, and with other connected computers, over a network 1713. Each computer 1700 can communicate with the other connected computers over the network 1713 through a network interface 1714 that enables communication over a connection 1716 between the network and the computer.

[0243] The computer 1700 operates under control of programming steps that are temporarily stored in the memory 1708 in accordance with conventional computer construction. When the programming steps are executed by the CPU 1702, the pertinent system components perform their respective functions. Thus, the programming steps implement the functionality of the system as described above. The programming steps can be received from the DASD 1707, through the program product reader 1712, or through the network connection 1716. The storage drive 1710 can receive a program product, read programming steps recorded thereon and transfer the programming steps into the memory 1708 for execution by the CPU 1702. As noted above, the program product storage device 1710 can comprise any one of multiple removable media having recorded computer-readable instructions, including magnetic floppy disks and CD-ROM storage discs. Other suitable program product storage devices can include magnetic tape and semiconductor memory chips. In this way, the processing steps necessary for operation can be embodied on a program product.

[0244] Alternatively, the program steps can be received into the operating memory 1708 over the network 1713. In the network method, the computer receives data including program steps into the memory 1708 through the network interface 1714 after network communication has been established over the network connection 1716 by well-known methods that will be understood by those skilled in the art without further explanation. The program steps are then executed by the CPU 1702 to implement the processing of the Garment Database system.

[0245] It should be understood that all of the computers of the system and can have a construction similar to that shown in FIG. 17. Details described with respect to the FIG. 17 computer 1700 will be understood to apply to all computers of the system 1700. This is indicated by multiple computers 1700 shown connected to the network 1713. Any one of the computers 1700 can have an alternative construction, so long as they can communicate with the other computers and support the functionality described herein.

[0246] FIG. 18 is a flow diagram that illustrates the processing steps performed using the computer illustrated in FIG. 17, to maintain and provide access to the databases, such as for identifying polymorphic genetic markers. In particular, the information contained in the database is stored in computers having a construction similar to that illustrated in FIG. 17. The first step for maintaining the database, as indicated in FIG. 18, is to identify healthy members of a population. As noted above, the population members are subjects that are selected only on the basis of

being healthy, and where the subjects are mammals, such as humans, they can be selected based upon apparent health and the absence of detectable infections. The step of identifying is represented by the flow diagram box numbered 1802.

[0247] The next step, represented by the flow diagram box numbered 1804, is to obtain identifying and historical information and data relating to the identified members of the population. The information and data comprise parameters for each of the population members, such as member age, ethnicity, sex, medical history, and ultimately genotypic information. Initially, the parameter information is obtained from a questionnaire answered by each member, from whom a body tissue or body fluid sample also is obtained. The step of entering and storing these parameters into the database of the computer is represented by the flow diagram box numbered 1806. As additional information about each population member and corresponding sample is obtained, this information can be inputted into the database and can serve as a sorting parameter.

[0248] In the next step, represented by the flow diagram box numbered 1808, the parameters of the members are associated with an indexer. This step can be executed as part of the database storage operation, such as when a new data record is stored according to the relational database structure and is automatically linked with other records according to that structure. The step 1806 also can be executed as part of a conventional data sorting or retrieval process, in which the database entries are searched according to an input search or indexing key value to determine attributes of the data. For example, such search and sort techniques can be used to follow the occurrence of known genetic markers and then determine if there is a correlation with diseases for which they have been implicated. Examples of this use are for assessing the frequencies of the p53 and Lipoprotein Lipase polymorphisms.

[0249] Such searching of the database also can be valuable for identifying one or more genetic markers whose frequency changes within the population as a function of age, ethnic group, sex, or some other criteria. This can allow the identification of previously unknown polymorphisms and, ultimately, identification of a gene or pathway involved in the onset and progression of disease.

[0250] In addition, the database can be used for taking an identified polymorphism and ascertaining whether it changes in frequency when the data are sorted according to a selected parameter.

[0251] In this way, the databases and methods provided herein permit, among other things, identification of components, particularly key components, of a disease process by understanding its genetic underpinnings, and also an understanding of processes, such as individual drug responses. The databases and methods provided herein also can be used in methods involving elucidation of pathological pathways, in developing new diagnostic assays, identifying new potential drug targets, and in identifying new drug candidates.

[0252] Morbidity and/or Early Mortality Associated Polymorphisms

[0253] A database containing information provided by a population of healthy blood donors who were not selected for any particular disease to can be used to identify poly-

morphisms and the alleles in which they are present, whose frequency decreases with age. These can represent morbidity susceptibility markers and genes.

[0254] Polymorphisms of the genome can lead to altered gene function, protein function or genome instability. To identify those polymorphisms which have a clinical relevance/utility is the goal of a world-wide scientific effort. It can be expected that the discovery of such polymorphisms will have a fundamental impact on the identification and development of novel drug compounds to cure diseases. The strategy to identify valuable polymorphisms is cumbersome and dependent upon the availability of many large patient and control cohorts to show disease association. In particular, genes that cause a general risk of the population to suffer from any disease (morbidity susceptibility genes) will escape these case/control studies entirely.

[0255] Here described is a screening strategy to identify morbidity susceptibility genes underlying a variety of different diseases. The definition of a morbidity susceptibility gene is a gene that is expressed in many different cell types or tissues (housekeeping gene) and its altered function can facilitate the expression of a clinical phenotype caused by disease-specific susceptibility genes that are involved in a pathway specific for this disorder. In other words, these morbidity susceptibility genes predispose people to develop a distinct disease according to their genetic make-up for this disease.

[0256] Candidates for morbidity susceptibility genes can be found at the bottom level of pathways involving transcription, translation, heat-shock proteins, protein trafficking, DNA repair, assembly systems for subcellular structures (e.g. mitochondria, peroxysomes and other cellular microbodies), receptor signaling cascades, immunology, etc. Those pathways control the quality of life at the cellular level as well as for the entire organism. Mutations/polymorphisms located in genes encoding proteins for those pathways can reduce the fitness of cells and make the organism more susceptible to express the clinical phenotype caused by the action of a disease-specific susceptibility gene. Therefore, these morbidity susceptibility genes can be potentially involved in a whole variety of different complex diseases if not in all. Disease-specific susceptibility genes are involved in pathways that can be considered as disease-specific pathways like glucose-, lipid, hormone metabolism, etc.

[0257] The exemplified method permit, among other things, identification of genes and/or gene products involved in a man's general susceptibility to morbidity and/or mortality; use of these genes and/or gene products in studies to elucidate the genetic underpinnings of human diseases; use of these genes and/or gene products in combinatorial statistical analyses without or together with disease-specific susceptibility genes; use of these genes and/or gene products to predict penetrance of disease susceptibility genes; use of these genes and/or gene products in predisposition and/or acute medical diagnostics and use of these genes and/or gene products to develop drugs to cure diseases and/or to extend the life span of humans.

[0258] SCREENING PROCESS

[0259] The healthy population stratified by age, gender and ethnicity, etc. is a very efficient and a universal screening tool for morbidity associated genes. Changes of allelic

frequencies in the young compared to the old population are expected to indicate putative morbidity susceptibility genes. Individual samples of this healthy population base can be pooled to further increase the throughput. In an experiment, pools of young and old Caucasian females and males were applied to screen more than 400 randomly chosen single nucleotide polymorphisms located in many different genes. Candidate polymorphisms were identified if the allelic difference was greater than 8% between young and old for both or only one of the genders. The initial results were assayed again in at least one independent subsequent experiments. Repeated experiments are necessary to recognize unstable biochemical reactions, which occur with a frequency of about 2-3% and can mimic age-related allelic frequency differences. Average frequency differences and standard deviations are calculated after successful reproducibility of initial results. The final allelic frequency is then compared to a reference population of Caucasian CEPH sample pool. The result should show similar allelic frequencies in the young Caucasian population. Subsequently, the exact allele frequencies of candidates including genotype information were obtained by analyzing all individual samples. This procedure is straight forward with regard to time and cost. It enables the screening of an enormous number of SNPs. So far, several markers with a highly significant association to age were identified and described below.

[0260] In general at least 5 individuals in a stratified population should to be screened to produce statistically significant results. The frequency of the allele is determined for an age stratified population. Chi square analysis is then performed on the allelic frequencies to determine if the difference between age groups is statistically significant. A p value less than of 0.1 is considered to represent a statistically significant difference. Typically the p value should be less than 0.05.

[0261] Clinical Trials

[0262] The identification of markers whose frequency in a population decreases with age also allows for better designed and balanced clinical trials. Currently, if a clinical trial utilizes a marker as a significant endpoint in a study and the marker disappears with age, then the results of the study can be inaccurate. By using methods provided herein, it can be ascertained that if a marker decreases in frequency with age. This information can be considered and controlled when designing the study. For, example, an age independent marker could be substituted in its place.

[0263] The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

EXAMPLE 1

[0264] This example describes the use of a database containing information provided by a population of healthy blood donors who were not selected for any particular disease to determine the distribution of allelic frequencies of known genetic markers with age and by sex in a Caucasian subpopulation of the database. The results described in this example demonstrate that a disease-related genetic marker or polymorphism can be identified by sorting a healthy database by a parameter or parameters, such as age, sex and ethnicity.

[0265] Generating a Database

[0266] Blood was obtained by venous puncture from human subjects who met blood bank criteria for donating blood. The blood samples were preserved with EDTA at pH 8.0 and labeled. Each donor provided information such as age, sex, ethnicity, medical history and family medical history. Each sample was labeled with a barcode representing identifying information. A database was generated by entering, for each donor, the subject identifier and information corresponding to that subject into the memory of a computer storage medium using commercially available software, e.g., Microsoft Access.

[0267] Model Genetic Markers

[0268] The frequencies of polymorphisms known to be associated at some level with disease were determined in a subpopulation of the subjects represented in the database. These known polymorphisms occur in the p21, p53 and Lipoprotein Lipase genes. Specifically, the N291S polymorphism (N291S) of the Lipoprotein Lipase gene, which results in a substitution of a serine for an asparagine at amino acid codon 291, leads to reduced levels of high density lipoprotein cholesterol (HDL-C) that is associated with an increased risk of males for arteriosclerosis and in particular myocardial infarction (see, Reymer et al. (1995) *Nature Genetics* 10:28-34).

[0269] The p53 gene encodes a cell cycle control protein that assesses DNA damage and acts as a transcription factor regulating genes that control cell growth, DNA repair and apoptosis (programmed cell death). Mutations in the p53 gene have been found in a wide variety of different cancers, including different types of leukemia, with varying frequency. The loss of normal p53 function results in genomic instability an uncontrolled cell growth. A polymorphism that has been identified in the p53 gene, i.e., the R72P allele, results in the substitution of a proline for an arginine at amino acid codon 72 of the gene.

[0270] The p21 gene encodes a cyclin-dependent kinase inhibitor associated with G1 phase arrest of normal cells. Expression of the p21 gene triggers apoptosis. Polymorphisms of the p21 gene have been associated with Wilms' tumor, a pediatric kidney cancer. One polymorphism of the p21 gene, the S31R polymorphism, results in a substitution of an arginine for a serine at amino acid codon 31.

[0271] Database Analysis

Sorting of Subjects According to Specific Parameters

[0272] The genetic polymorphisms were profiled within segments of the Caucasian subpopulation of the sample bank. For p53 profiling, the genomic DNA isolated from blood from a total of 1277 Caucasian subjects age 18-59 years and 457 Caucasian subjects age 60-79 years was analyzed. For p21 profiling, the genomic DNA isolated from blood from a total of 910 Caucasian subjects age 18-49 years and 824 Caucasian subjects age 50-79 years was analyzed. For lipoprotein lipase gene profiling, the genomic DNA from a total of 1464 Caucasian females and 1470 Caucasian males under 60 years of age and a total of 478 Caucasian females and 560 Caucasian males over 60 years of age was analyzed.

Isolation and Analysis of Genomic DNA

[0273] Genomic DNA was isolated from blood samples obtained from the individuals. Ten milliliters of whole blood from each individual was centrifuged at 2000×g. One milliliter of the buffy coat was added to 9 ml of 155 mM NH₄Cl, 10 mM KHCO₃, and 0.1 mM Na₂EDTA, incubated 10 min at room temperature and centrifuged for 10 min at 2000×g. The supernatant was removed, and the white cell pellet was washed in 155 mM NH₄Cl, 10 mM KHCO₃ and 0.1 mM Na₂EDTA and resuspended in 4.5 ml of 50 mM Tris, 5 mM EDTA and 1% SDS. Proteins were precipitated from the cell lysate by 6 mM ammonium acetate, pH 7.3, and then separated from the nucleic acids by centrifugation at 3000×g. The nucleic acid was recovered from the supernatant by the addition of an equal volume of 100% isopropanol and centrifugation at 2000×g. The dried nucleic acid pellet was hydrated in 10 mM Tris, pH 7.6, and 1 mM Na₂EDTA and stored at 4° C.

[0274] Assays of the genomic DNA to determine the presence or absence of the known genetic markers were developed using the BiomassPROBE™ detection method (primer oligo base extension) reaction. This method uses a single detection primer followed by an oligonucleotide extension step to give products, which can be readily resolved by mass spectrometry, and, in particular, MALDI-TOF mass spectrometry. The products differ in length depending on the presence or absence of a polymorphism. In this method, a detection primer anneals adjacent to the site of a variable nucleotide or sequence of nucleotides, and the primer is extended using a DNA polymerase in the presence of one or more dideoxynTPs and, optionally, one or more deoxynTPs. The resulting products are resolved by MALDI-TOF mass spectrometry. The mass of the products as measured by MALDI-TOF mass spectrometry makes possible the determination of the nucleotide(s) present at the variable site.

[0275] First, each of the Caucasian genomic DNA samples was subjected to nucleic acid amplification using primers corresponding to sites 5' and 3' of the polymorphic sites of the p21 (S31R allele), p53 (R72P allele) and Lipoprotein Lipase (N291S allele) genes. One primer in each primer pair was biotinylated to permit immobilization of the amplification product to a solid support. Specifically, the polymerase chain reaction primers used for amplification of the relevant segments of the p21, p53 and lipoprotein lipase genes are shown below: US4p21c31-2F (SEQ ID NO: 9) and US5p21-2R (SEQ ID NO: 10) for p21 gene amplification; US4-p53-ex4-F (also shown as p53-ex4US4 (SEQ ID NO: 2)) and US5-p53/2-4R (also shown as US5P53/4R (SEQ ID NO: 3)) for p53 gene amplification; and US4-LPL-F2 (SEQ ID NO: 16) and US5-LPL-R2 (SEQ ID NO: 17) for lipoprotein lipase gene amplification.

[0276] Amplification of the respective DNA sequences was conducted according to standard protocols. For example, primers can be used in a concentration of 8 pmol. The reaction mixture (e.g., total volume 50 µl) can contain Taq-polymerase including 10×buffer and dTNPs. Cycling conditions for polymerase chain reaction amplification can typically be initially 5 min. at 95° C., followed by 1 min. at 94° C., 45 sec at 53° C., and 30 sec at 72° C. for 40 cycles with a final extension time of 5 min at 72° C. Amplification products can be purified by using Qiagen's PCR purification

kit (No. 28106) according to manufacturer's instructions. The elution of the purified products from the column can be done in 50 μ l TE-buffer (10 mM Tris, 1 mM EDTA, pH 7.5).

[0277] The purified amplification products were immobilized via a biotin-avidin linkage to streptavidin-coated beads and the double-stranded DNA was denatured. A detection primer was then annealed to the immobilized DNA using conditions such as, for example, the following: 50 μ l annealing buffer (20 mM Tris, 10 mM KCl, 10 mM $(\text{NH}_4)_2\text{SO}_4$, 2 mM MgSO_4 , 1% Triton X-100, pH 8) at 50° C. for 10 min, followed by washing of the beads three times with 200 μ l washing buffer (40 mM Tris, 1 mM EDTA, 50 mM NaCl, 0.1% Tween 20, pH 8.8) and once in 200 μ l TE buffer.

[0278] The PROBE extension reaction was performed, for example, by, using some components of the DNA sequencing kit from USB (No. 70770) and dNTPs or ddNTPs from Pharmacia. An exemplary protocol could include a total reaction volume of 45 μ l, containing of 21 μ l water, 6 μ l Sequenase-buffer, 3 μ l 10 mM DTT solution, 4.5 μ l, 0.5 mM of three dNTPs, 4.5 μ l, 2 mM the missing one ddNTP, 5.5 μ l glycerol enzyme dilution buffer, 0.25 μ l Sequenase 2.0, and 0.25 pyrophosphatase. The reaction can then be pipetted on ice and incubated for 15 min at room temperature and for 5 min at 37° C. The beads can be washed three times with 200 μ l washing buffer and once with 60 μ l of a 70 mM NH_4 -Citrate solution.

[0279] The DNA was denatured to release the extended primers from the immobilized template. Each of the resulting extension products was separately analyzed by MALDI-TOF mass spectrometry using 3-hydroxypicolinic acid (3-HPA) as matrix and a UV laser.

[0280] Specifically, the primers used in the PROBE reactions are as shown below: P21/31-3 (SEQ ID NO: 12) for PROBE analysis of the p21 polymorphic site; P53/72 (SEQ ID NO: 4) for PROBE analysis of the p53 polymorphic site;

and LPL-2 for PROBE analysis of the lipoprotein lipase gene polymorphic site. In the PROBE analysis of the p21 polymorphic site, the extension reaction was performed using dideoxy-C. The products resulting from the reaction conducted on a "wild-type" allele template (wherein codon 31 encodes a serine) and from the reaction conducted on a polymorphic S31R allele template (wherein codon 31 encodes an arginine) are shown below and designated as P21/31-3 Ser (wt) (SEQ ID NO: 13) and P21/31-3 Arg (SEQ ID NO: 14), respectively. The masses for each product as can be measured by MALDI-TOF mass spectrometry are also provided (i.e., 4900.2 Da for the wild-type product and 5213.4 Da for the polymorphic product).

[0281] In the PROBE analysis of the p53 polymorphic site, the extension reaction was performed using dideoxy-C. The products resulting from the reaction conducted on a "wild-type" allele template (wherein codon 72 encodes an arginine) and from the reaction conducted on a polymorphic R72P allele template (wherein codon 72 encodes a proline) are shown below and designated as Cod72 G Arg (wt) and Cod72 C Pro, respectively. The masses for each product as can be measured by MALDI-TOF mass spectrometry are also provided (i.e., 5734.8 Da for the wild-type product and 5405.6 Da for the polymorphic product).

[0282] In the PROBE analysis of the lipoprotein lipase gene polymorphic site, the extension reaction was performed using a mixture of ddA and ddT. The products resulting from the reaction conducted on a "wild-type" allele template (wherein codon 291 encodes an asparagine) and from the reaction conducted on a polymorphic N291S allele template (wherein codon 291 encodes a serine) are shown below and designated as 291Asn and 291Ser, respectively. The masses for each product as can be measured by MALDI-TOF mass spectrometry are also provided (i.e., 6438.2 Da for the wild-type product and 6758.4 Da for the polymorphic product).

P53-1 (R72P)	
PCR Product length: 407 bp	
US4-p53-ex4-F	(SEQ ID NO: 1)
ctg aggacctggt cctctgaactg	
ctctttttcag ccatctacagtcctcccttgcctgcccaaga aat	
ggatgatttgatgctgt	
cccgggacga tattgaacaa tggttcactg aagaccacag tccagatgaa gctc-	
ccagaa	
P53/72	72R
tgcagagggctgctccccc gtggccccctg caccagcagc tctacacog gcggc-	
cctg	
c 72P	
caccagcccc ctctctggccc ctgtcatctt ctgtcccttc ccagaaaacc tac-	
caggga	
gtacaggttt ccgtctgggc ttcttgcatc ctgggacagc caagtctgtg acttg-	
cacgg	
tcagttgccc tgaggggctg gcttccatga gacttcaa	
US5-p53/2-4R	

[0283]

Primers (SEQ ID NOs: 2-4)	
p53-ex4FUS4	<u>cccagtcacgacggttgtaaaacgc</u> tga gga cct ggt cct ctg ac
US5P53/4R	<u>agcggataacaatttcacacacaggt</u> tga agt ctc atg gaa gcc
P53/72	gcc aga ggc tgc tcc cc

[0284]

Masses				
Allele	Product Termination: ddC	SEQ Len-#	gth	Mass
P53/72	gccagaggctgctcccc	5	17	5132.4
Cod72 G Arg (wt)	gccagaggctgctccccgc	6	19	5734.8
Cod72 C Pro	gccagaggctgctcccc	7	18	5405.6

[0285] Biotinylated US5 primer is used in the PCR amplification.

[0286] LPL-1 (N291S)

[0287] Amino acid exchange asparagine to serine at codon 291 of the lipoprotein lipase gene.

PCR Product length: 251 bp (SEQ ID NO: 15)	
US4-LPL-F2	(SEQ ID NO: 16)
<u>gcgctccatt catctcttcca</u> tcgactctct gttgaatgaa gaaaatccaa gtaag-gccta	
cagggtgcagt tccaaggaag cctttgagaa agggctctgc ttgagttgta gaaa-gaaccg	
LPL-2	291N
ctgcaacaatctgggctatgagatcaataa agtcagagcc aaaagaagca gcaaaat-gta	
	g 291S
cctgaagact cgttctcaga tgcgc	
US4-LPL-R2	

[0288]

Primers (SEQ ID NOs: 16-18):	
US4-LPL-F2	<u>cccagtcacgacggttgtaaaacgg</u> cgc tcc att cat ctc ttc
US5-LPL-R2	<u>agcggataacaatttcacacacagg</u> ggc atc tga gaa cga gtc
LPL-2	caa tct ggg cta tga gat ca

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tcggggccgg ttgctga atg agg gga gcc ggg ccc tcc ccg cgc cag tcc      170
           Met Arg Gly Ala Gly Pro Ser Pro Arg Gln Ser
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ccc cgc acc ctc cgt ccc gac ccg ggc ccc gcc atg tcc ttc ttc cgg      218
Pro Arg Thr Leu Arg Pro Asp Pro Gly Pro Ala Met Ser Phe Phe Arg
           15             20             25

cgg aaa gtg aaa ggc aaa gaa caa gag aag acc tca gat gtg aag tcc      266
Arg Lys Val Lys Gly Lys Glu Gln Lys Thr Ser Asp Val Lys Ser
           30             35             40

att aaa gct tca ata tcc gta cat tcc cca caa aaa agc act aaa aat      314
Ile Lys Ala Ser Ile Ser Val His Ser Pro Gln Lys Ser Thr Lys Asn
           45             50             55

cat gcc ttg ctg gag gct gca gga cca agt cat gtt gca atc aat gcc      362
His Ala Leu Leu Glu Ala Ala Gly Pro Ser His Val Ala Ile Asn Ala
           60             65             70             75

att tct gcc aac atg gac tcc ttt tca agt agc agg aca gcc aca ctt      410
Ile Ser Ala Asn Met Asp Ser Phe Ser Ser Ser Arg Thr Ala Thr Leu
           80             85             90

aag aag cag cca agc cac atg gag gct gct cat ttt ggt gac ctg ggc      458
Lys Lys Gln Pro Ser His Met Glu Ala Ala His Phe Gly Asp Leu Gly
           95             100             105

aga tct tgt ctg gac tac cag act caa gag acc aaa tca agc ctt tct      506
Arg Ser Cys Leu Asp Tyr Gln Thr Gln Glu Thr Lys Ser Ser Leu Ser
           110             115             120

aag acc ctt gaa caa gtc ttg cac gac act att gtc ctc cct tac ttc      554
Lys Thr Leu Glu Gln Val Leu His Asp Thr Ile Val Leu Pro Tyr Phe
           125             130             135

att caa ttc atg gaa ctt cgg cga atg gag cat ttg gtg aaa ttt tgg      602
Ile Gln Phe Met Glu Leu Arg Arg Met Glu His Leu Val Lys Phe Trp
           140             145             150             155

tta gag gct gaa agt ttt cat tca aca act tgg tgg cga ata aga gca      650
Leu Glu Ala Glu Ser Phe His Ser Thr Thr Trp Ser Arg Ile Arg Ala
           160             165             170

cac agt cta aac aca atg aag cag agc tca ctg gct gag cct gtc tot      698
His Ser Leu Asn Thr Met Lys Gln Ser Ser Leu Ala Glu Pro Val Ser
           175             180             185

cca tct aaa aag cat gaa act aca gcg tct ttt tta act gat tct ctt      746
Pro Ser Lys Lys His Glu Thr Thr Ala Ser Phe Leu Thr Asp Ser Leu
           190             195             200

gat aag aga ttg gag gat tct ggc tca gca cag ttg ttt atg act cat      794
Asp Lys Arg Leu Glu Asp Ser Gly Ser Ala Gln Leu Phe Met Thr His
           205             210             215

tca gaa gga att gac ctg aat aat aga act aac agc act cag aat cac      842
Ser Glu Gly Ile Asp Leu Asn Asn Arg Thr Asn Ser Thr Gln Asn His
           220             225             230             235

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ttg ctg ctt tcc cag gaa tgt gac agt gcc cat tct ctc cgt ctt gaa	890
Leu Leu Leu Ser Gln Glu Cys Asp Ser Ala His Ser Leu Arg Leu Glu	
240 245 250	
atg gcc aga gca gga act cac caa gtt tcc atg gaa acc caa gaa tct	938
Met Ala Arg Ala Gly Thr His Gln Val Ser Met Glu Thr Gln Glu Ser	
255 260 265	
tcc tct aca ctt aca gta gcc agt aga aat agt ccc gct tct cca cta	986
Ser Ser Thr Leu Thr Val Ala Ser Arg Asn Ser Pro Ala Ser Pro Leu	
270 275 280	
aaa gaa ttg tca gga aaa cta atg aaa agt ata gaa caa gat gca gtg	1034
Lys Glu Leu Ser Gly Lys Leu Met Lys Ser Ile Glu Gln Asp Ala Val	
285 290 295	
aat act ttt acc aaa tat ata tct cca gat gct gct aaa cca ata cca	1082
Asn Thr Phe Thr Lys Tyr Ile Ser Pro Asp Ala Ala Lys Pro Ile Pro	
300 305 310 315	
att aca gaa gca atg aga aat gac atc ata gca agg att tgt gga gaa	1130
Ile Thr Glu Ala Met Arg Asn Asp Ile Ile Ala Arg Ile Cys Gly Glu	
320 325 330	
gat gga cag gtg gat ccc aac tgt ttc gtt ttg gca cag tcc ata gtc	1178
Asp Gly Gln Val Asp Pro Asn Cys Phe Val Leu Ala Gln Ser Ile Val	
335 340 345	
ttt agt gca atg gag caa gag cac ttt agt gag ttt ctg cga agt cac	1226
Phe Ser Ala Met Glu Gln Glu His Phe Ser Glu Phe Leu Arg Ser His	
350 355 360	
cat ttc tgt aaa tac cag att gaa gtg ctg acc agt gga act gtt tac	1274
His Phe Cys Lys Tyr Gln Ile Glu Val Leu Thr Ser Gly Thr Val Tyr	
365 370 375	
ctg gct gac att ctc ttc tgt gag tca gcc ctc ttt tat ttc tct gag	1322
Leu Ala Asp Ile Leu Phe Cys Glu Ser Ala Leu Phe Tyr Phe Ser Glu	
380 385 390 395	
tac atg gaa aaa gag gat gca gtg aat atc tta caa ttc tgg ttg gca	1370
Tyr Met Glu Lys Glu Asp Ala Val Asn Ile Leu Gln Phe Trp Leu Ala	
400 405 410	
gca gat aac ttc cag tct cag ctt gct gcc aaa aag ggg caa tat gat	1418
Ala Asp Asn Phe Gln Ser Gln Leu Ala Ala Lys Lys Gly Gln Tyr Asp	
415 420 425	
gga cag gag gca cag aat gat gcc atg att tta tat gac aag tac ttc	1466
Gly Gln Glu Ala Gln Asn Asp Ala Met Ile Leu Tyr Asp Lys Tyr Phe	
430 435 440	
tcc ctc caa gcc aca cat cct ctt gga ttt gat gat gtt gta cga tta	1514
Ser Leu Gln Ala Thr His Pro Leu Gly Phe Asp Asp Val Val Arg Leu	
445 450 455	
gaa att gaa tcc aat atc tgc agg gaa ggt ggg cca ctc ccc aac tgt	1562
Glu Ile Glu Ser Asn Ile Cys Arg Glu Gly Gly Pro Leu Pro Asn Cys	
460 465 470 475	
ttc aca aot cca tta cgt cag gcc tgg aca acc atg gag aag gtc ttt	1610
Phe Thr Thr Pro Leu Arg Gln Ala Trp Thr Thr Met Glu Lys Val Phe	
480 485 490	
ttg cct gcc ttt ctg tcc agc aat ctt tat tat aaa tat ttg aat gat	1658
Leu Pro Gly Phe Leu Ser Ser Asn Leu Tyr Tyr Lys Tyr Leu Asn Asp	
495 500 505	
ctc atc cat tcg gtt cga gga gat gaa ttt ctg gcc ggg aac gtg tcg	1706
Leu Ile His Ser Val Arg Gly Asp Glu Phe Leu Gly Gly Asn Val Ser	
510 515 520	
ccg act gct cct gcc tct gtt gcc cct cct gat gag tct cac cca ggg	1754
Pro Thr Ala Pro Gly Ser Val Gly Pro Pro Asp Glu Ser His Pro Gly	
525 530 535	

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agt tct gac agc tct gcg tct cag tcc agt gtg aaa aaa gcc agt att      1802
Ser Ser Asp Ser Ser Ala Ser Gln Ser Ser Val Lys Lys Ala Ser Ile
540                      545                      550                      555

aaa ata ctg aaa aat ttt gat gaa gcg ata att gtg gat gcg gca agt      1850
Lys Ile Leu Lys Asn Phe Asp Glu Ala Ile Ile Val Asp Ala Ala Ser
                    560                      565                      570

ctg gat cca gaa tct tta tat caa cgg aca tat gcc ggg aag atg aca      1898
Leu Asp Pro Glu Ser Leu Tyr Gln Arg Thr Tyr Ala Gly Lys Met Thr
                    575                      580                      585

ttt gga aga gtg agt gac ttg ggg caa ttc atc cgg gaa tct gag cct      1946
Phe Gly Arg Val Ser Asp Leu Gly Gln Phe Ile Arg Glu Ser Glu Pro
                    590                      595                      600

gaa cct gat gta agg aaa tca aaa gga tcc atg ttc tca caa gct atg      1994
Glu Pro Asp Val Arg Lys Ser Lys Gly Ser Met Phe Ser Gln Ala Met
                    605                      610                      615

aag aaa tgg gtg caa gga aat act gat gag gcc cag gaa gag cta gct      2042
Lys Lys Trp Val Gln Gly Asn Thr Asp Glu Ala Gln Glu Glu Leu Ala
620                      625                      630                      635

tgg aag att gct aaa atg ata gtc agt gac att atg cag cag gct cag      2090
Trp Lys Ile Ala Lys Met Ile Val Ser Asp Ile Met Gln Gln Ala Gln
                    640                      645                      650

tat gat caa cgg tta gag aaa tct aca aag tta tga ctcaaaactt      2136
Tyr Asp Gln Pro Leu Glu Lys Ser Thr Lys Leu *
                    655                      660

gagataaagg aaatctgctt gtgaaaaata agagaacttt tttcccttgg ttggattctt      2196

caacacagcc aatgaaaaca gcactatatt tctgatctgt cactgttggt tccagggaga      2256

gaatggggag acaatcctag gacttccacc ctaatgcagt tacctgtagg gcataattgg      2316

atggcacatg atgtttcaca cagtgaggag tctttaaagg ttaccaa      2363

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<210> SEQ ID NO 32

<211> LENGTH: 662

<212> TYPE: PRT

<213> ORGANISM: Homo Sapien

<400> SEQUENCE: 32

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Met Arg Gly Ala Gly Pro Ser Pro Arg Gln Ser Pro Arg Thr Leu Arg
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Pro Asp Pro Gly Pro Ala Met Ser Phe Phe Arg Arg Lys Val Lys Gly
                20              25              30

Lys Glu Gln Glu Lys Thr Ser Asp Val Lys Ser Ile Lys Ala Ser Ile
                35              40              45

Ser Val His Ser Pro Gln Lys Ser Thr Lys Asn His Ala Leu Leu Glu
 50              55              60

Ala Ala Gly Pro Ser His Val Ala Ile Asn Ala Ile Ser Ala Asn Met
65              70              75              80

Asp Ser Phe Ser Ser Ser Arg Thr Ala Thr Leu Lys Lys Gln Pro Ser
                85              90              95

His Met Glu Ala Ala His Phe Gly Asp Leu Gly Arg Ser Cys Leu Asp
100              105              110

Tyr Gln Thr Gln Glu Thr Lys Ser Ser Leu Ser Lys Thr Leu Glu Gln
115              120              125

Val Leu His Asp Thr Ile Val Leu Pro Tyr Phe Ile Gln Phe Met Glu
130              135              140

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Leu	Arg	Arg	Met	Glu	His	Leu	Val	Lys	Phe	Trp	Leu	Glu	Ala	Glu	Ser	145	150	155	160
Phe	His	Ser	Thr	Thr	Trp	Ser	Arg	Ile	Arg	Ala	His	Ser	Leu	Asn	Thr	165	170	175	
Met	Lys	Gln	Ser	Ser	Leu	Ala	Glu	Pro	Val	Ser	Pro	Ser	Lys	Lys	His	180	185	190	
Glu	Thr	Thr	Ala	Ser	Phe	Leu	Thr	Asp	Ser	Leu	Asp	Lys	Arg	Leu	Glu	195	200	205	
Asp	Ser	Gly	Ser	Ala	Gln	Leu	Phe	Met	Thr	His	Ser	Glu	Gly	Ile	Asp	210	215	220	
Leu	Asn	Asn	Arg	Thr	Asn	Ser	Thr	Gln	Asn	His	Leu	Leu	Leu	Ser	Gln	225	230	235	240
Glu	Cys	Asp	Ser	Ala	His	Ser	Leu	Arg	Leu	Glu	Met	Ala	Arg	Ala	Gly	245	250	255	
Thr	His	Gln	Val	Ser	Met	Glu	Thr	Gln	Glu	Ser	Ser	Ser	Thr	Leu	Thr	260	265	270	
Val	Ala	Ser	Arg	Asn	Ser	Pro	Ala	Ser	Pro	Leu	Lys	Glu	Leu	Ser	Gly	275	280	285	
Lys	Leu	Met	Lys	Ser	Ile	Glu	Gln	Asp	Ala	Val	Asn	Thr	Phe	Thr	Lys	290	295	300	
Tyr	Ile	Ser	Pro	Asp	Ala	Ala	Lys	Pro	Ile	Pro	Ile	Thr	Glu	Ala	Met	305	310	315	320
Arg	Asn	Asp	Ile	Ile	Ala	Arg	Ile	Cys	Gly	Glu	Asp	Gly	Gln	Val	Asp	325	330	335	
Pro	Asn	Cys	Phe	Val	Leu	Ala	Gln	Ser	Ile	Val	Phe	Ser	Ala	Met	Glu	340	345	350	
Gln	Glu	His	Phe	Ser	Glu	Phe	Leu	Arg	Ser	His	His	Phe	Cys	Lys	Tyr	355	360	365	
Gln	Ile	Glu	Val	Leu	Thr	Ser	Gly	Thr	Val	Tyr	Leu	Ala	Asp	Ile	Leu	370	375	380	
Phe	Cys	Glu	Ser	Ala	Leu	Phe	Tyr	Phe	Ser	Glu	Tyr	Met	Glu	Lys	Glu	385	390	395	400
Asp	Ala	Val	Asn	Ile	Leu	Gln	Phe	Trp	Leu	Ala	Ala	Asp	Asn	Phe	Gln	405	410	415	
Ser	Gln	Leu	Ala	Ala	Lys	Lys	Gly	Gln	Tyr	Asp	Gly	Gln	Glu	Ala	Gln	420	425	430	
Asn	Asp	Ala	Met	Ile	Leu	Tyr	Asp	Lys	Tyr	Phe	Ser	Leu	Gln	Ala	Thr	435	440	445	
His	Pro	Leu	Gly	Phe	Asp	Asp	Val	Val	Arg	Leu	Glu	Ile	Glu	Ser	Asn	450	455	460	
Ile	Cys	Arg	Glu	Gly	Gly	Pro	Leu	Pro	Asn	Cys	Phe	Thr	Thr	Pro	Leu	465	470	475	480
Arg	Gln	Ala	Trp	Thr	Thr	Met	Glu	Lys	Val	Phe	Leu	Pro	Gly	Phe	Leu	485	490	495	
Ser	Ser	Asn	Leu	Tyr	Tyr	Lys	Tyr	Leu	Asn	Asp	Leu	Ile	His	Ser	Val	500	505	510	
Arg	Gly	Asp	Glu	Phe	Leu	Gly	Gly	Asn	Val	Ser	Pro	Thr	Ala	Pro	Gly	515	520	525	
Ser	Val	Gly	Pro	Pro	Asp	Glu	Ser	His	Pro	Gly	Ser	Ser	Asp	Ser	Ser	530	535	540	
Ala	Ser	Gln	Ser	Ser	Val	Lys	Lys	Ala	Ser	Ile	Lys	Ile	Leu	Lys	Asn				

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545	550	555	560
Phe Asp Glu Ala Ile Ile Val Asp Ala Ala Ser Leu Asp Pro Glu Ser			
	565	570	575
Leu Tyr Gln Arg Thr Tyr Ala Gly Lys Met Thr Phe Gly Arg Val Ser			
	580	585	590
Asp Leu Gly Gln Phe Ile Arg Glu Ser Glu Pro Glu Pro Asp Val Arg			
	595	600	605
Lys Ser Lys Gly Ser Met Phe Ser Gln Ala Met Lys Lys Trp Val Gln			
	610	615	620
Gly Asn Thr Asp Glu Ala Gln Glu Glu Leu Ala Trp Lys Ile Ala Lys			
	625	630	635
Met Ile Val Ser Asp Ile Met Gln Gln Ala Gln Tyr Asp Gln Pro Leu			
	645	650	655
Glu Lys Ser Thr Lys Leu			
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ccactcccg aagaagggtc ccttttcgcg ctagtgcage ggccctctg gacccggaag			120
tcggggccgg ttgctga atg agg gga gcc ggg ccc tcc ccg cgc cag tcc			170
Met Arg Gly Ala Gly Pro Ser Pro Arg Gln Ser	1	5	10
ccc cgc acc ctc cgt ccc gac ccg ggc ccc gcc atg tcc ttc ttc cgg			218
Pro Arg Thr Leu Arg Pro Asp Pro Gly Pro Ala Met Ser Phe Phe Arg	15	20	25
cgg aaa gtg aaa ggc aaa gaa caa gag aag acc tca gat gtg aag tcc			266
Arg Lys Val Lys Gly Lys Glu Gln Glu Lys Thr Ser Asp Val Lys Ser	30	35	40
att aaa gct tca ata tcc gta cat tcc cca caa aaa agc act aaa aat			314
Ile Lys Ala Ser Ile Ser Val His Ser Pro Gln Lys Ser Thr Lys Asn	45	50	55
cat gcc ttg ctg gag gct gca gga cca agt cat gtt gca atc aat gcc			362
His Ala Leu Leu Glu Ala Ala Gly Pro Ser His Val Ala Ile Asn Ala	60	65	75
att tct gcc aac atg gac tcc ttt tca agt agc agg aca gcc aca ctt			410
Ile Ser Ala Asn Met Asp Ser Phe Ser Ser Ser Arg Thr Ala Thr Leu	80	85	90
aag aag cag cca agc cac atg gag gct got cat ttt ggt gac ctg ggc			458
Lys Lys Gln Pro Ser His Met Glu Ala Ala His Phe Gly Asp Leu Gly	95	100	105
aga tct tgt ctg gac tac cag act caa gag acc aaa tca agc ctt tct			506
Arg Ser Cys Leu Asp Tyr Gln Thr Gln Glu Thr Lys Ser Ser Leu Ser	110	115	120
aag acc ctt gaa caa gtc ttg cac gac act att gtc ctc cct tac ttc			554

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Lys	Thr	Leu	Glu	Gln	Val	Leu	His	Asp	Thr	Ile	Val	Leu	Pro	Tyr	Phe	
125						130					135					
att	caa	ttc	atg	gaa	ctt	cgg	cga	atg	gag	cat	ttg	gtg	aaa	ttt	tgg	602
Ile	Gln	Phe	Met	Glu	Leu	Arg	Arg	Met	Glu	His	Leu	Val	Lys	Phe	Trp	
140					145					150					155	
tta	gag	gct	gaa	agt	ttt	cat	tca	aca	act	tgg	tcg	cga	ata	aga	gca	650
Leu	Glu	Ala	Glu	Ser	Phe	His	Ser	Thr	Thr	Trp	Ser	Arg	Ile	Arg	Ala	
				160					165					170		
cac	agt	cta	aac	aca	atg	aag	cag	agc	tca	ctg	gct	gag	cct	gtc	tct	698
His	Ser	Leu	Asn	Thr	Met	Lys	Gln	Ser	Ser	Leu	Ala	Glu	Pro	Val	Ser	
			175					180					185			
cca	tct	aaa	aag	cat	gaa	act	aca	gcg	tct	ttt	tta	act	gat	tct	ctt	746
Pro	Ser	Lys	Lys	His	Glu	Thr	Thr	Ala	Ser	Phe	Leu	Thr	Asp	Ser	Leu	
		190					195					200				
gat	aag	aga	ttg	gag	gat	tct	ggc	tca	gca	cag	ttg	ttt	atg	act	cat	794
Asp	Lys	Arg	Leu	Glu	Asp	Ser	Gly	Ser	Ala	Gln	Leu	Phe	Met	Thr	His	
	205					210					215					
tca	gaa	gga	att	gac	ctg	aat	aat	aga	act	aac	agc	act	cag	aat	cac	842
Ser	Glu	Gly	Ile	Asp	Leu	Asn	Asn	Arg	Thr	Asn	Ser	Thr	Gln	Asn	His	
	220				225					230					235	
ttg	ctg	ctt	tcc	cag	gaa	tgt	gac	agt	gcc	cat	tct	ctc	cgt	ctt	gaa	890
Leu	Leu	Leu	Ser	Gln	Glu	Cys	Asp	Ser	Ala	His	Ser	Leu	Arg	Leu	Glu	
				240					245					250		
atg	gcc	aga	gca	gga	act	cac	caa	gtt	tcc	atg	gaa	acc	caa	gaa	tct	938
Met	Ala	Arg	Ala	Gly	Thr	His	Gln	Val	Ser	Met	Glu	Thr	Gln	Glu	Ser	
			255					260					265			
tcc	tct	aca	ctt	aca	gta	gcc	agt	aga	aat	agt	ccc	gct	tct	cca	cta	986
Ser	Ser	Thr	Leu	Thr	Val	Ala	Ser	Arg	Asn	Ser	Pro	Ala	Ser	Pro	Leu	
		270					275					280				
aaa	gaa	ttg	tca	gga	aaa	cta	atg	aaa	agt	ata	gaa	caa	gat	gca	gtg	1034
Lys	Glu	Leu	Ser	Gly	Lys	Leu	Met	Lys	Ser	Ile	Glu	Gln	Asp	Ala	Val	
	285					290					295					
aat	act	ttt	acc	aaa	tat	ata	tct	cca	gat	gct	gct	aaa	cca	ata	cca	1082
Asn	Thr	Phe	Thr	Lys	Tyr	Ile	Ser	Pro	Asp	Ala	Ala	Lys	Pro	Ile	Pro	
	300				305					310					315	
att	aca	gaa	gca	atg	aga	aat	gac	atc	ata	gca	agg	att	tgt	gga	gaa	1130
Ile	Thr	Glu	Ala	Met	Arg	Asn	Asp	Ile	Ile	Ala	Arg	Ile	Cys	Gly	Glu	
				320					325					330		
gat	gga	cag	gtg	gat	ccc	aac	tgt	ttc	gtt	ttg	gca	cag	tcc	ata	gtc	1178
Asp	Gly	Gln	Val	Asp	Pro	Asn	Cys	Phe	Val	Leu	Ala	Gln	Ser	Ile	Val	
			335					340					345			
ttt	agt	gca	atg	gag	caa	gag	cac	ttt	agt	gag	ttt	ctg	cga	agt	cac	1226
Phe	Ser	Ala	Met	Glu	Gln	Glu	His	Phe	Ser	Glu	Phe	Leu	Arg	Ser	His	
		350					355					360				
cat	ttc	tgt	aaa	tac	cag	att	gaa	gtg	ctg	acc	agt	gga	act	gtt	tac	1274
His	Phe	Cys	Lys	Tyr	Gln	Ile	Glu	Val	Leu	Thr	Ser	Gly	Thr	Val	Tyr	
	365					370					375					
ctg	gct	gac	att	ctc	ttc	tgt	gag	tca	gcc	ctc	ttt	tat	tto	tct	gag	1322
Leu	Ala	Asp	Ile	Leu	Phe	Cys	Glu	Ser	Ala	Leu	Phe	Tyr	Phe	Ser	Glu	
					385					390					395	
tac	atg	gaa	aaa	gag	gat	gca	gtg	aat	atc	tta	caa	tto	tgg	ttg	gca	1370
Tyr	Met	Glu	Lys	Glu	Asp	Ala	Val	Asn	Ile	Leu	Gln	Phe	Trp	Leu	Ala	
				400					405					410		
gca	gat	aac	ttc	cag	tct	cag	ctt	gct	gcc	aaa	aag	ggg	caa	tat	gat	1418
Ala	Asp	Asn	Phe	Gln	Ser	Gln	Leu	Ala	Ala	Lys	Lys	Gly	Gln	Tyr	Asp	
				415				420					425			
gga	cag	gag	gca	cag	aat	gat	gcc	atg	att	tta	tat	gac	aag	tac	ttc	1466

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Gly	Gln	Glu	Ala	Gln	Asn	Asp	Ala	Met	Ile	Leu	Tyr	Asp	Lys	Tyr	Phe	
		430					435					440				
tcc	ctc	caa	gcc	aca	cat	cct	ctt	gga	ttt	gat	gat	gtt	gta	cga	tta	1514
Ser	Leu	Gln	Ala	Thr	His	Pro	Leu	Gly	Phe	Asp	Asp	Val	Val	Arg	Leu	
		445				450					455					
gaa	att	gaa	tcc	aat	atc	tgc	agg	gaa	ggt	ggg	cca	ctc	ccc	aac	tgt	1562
Glu	Ile	Glu	Ser	Asn	Ile	Cys	Arg	Glu	Gly	Gly	Pro	Leu	Pro	Asn	Cys	
460					465					470					475	
ttc	aca	act	cca	tta	cgt	cag	gcc	tgg	aca	acc	atg	gag	aag	gtc	ttt	1610
Phe	Thr	Thr	Pro	Leu	Arg	Gln	Ala	Trp	Thr	Thr	Met	Glu	Lys	Val	Phe	
				480					485					490		
ttg	cct	ggc	ttt	ctg	tcc	agc	aat	ctt	tat	tat	aaa	tat	ttg	aat	gat	1658
Leu	Pro	Gly	Phe	Leu	Ser	Ser	Asn	Leu	Tyr	Tyr	Lys	Tyr	Leu	Asn	Asp	
		495					500						505			
ctc	atc	cat	tgc	gtt	cga	gga	gat	gaa	ttt	ctg	ggc	ggg	aac	gtg	tgc	1706
Leu	Ile	His	Ser	Val	Arg	Gly	Asp	Glu	Phe	Leu	Gly	Gly	Asn	Val	Ser	
		510				515						520				
cgc	act	gct	cct	ggc	tct	gtt	ggc	cct	cct	gat	gag	tct	cac	cca	ggg	1754
Pro	Thr	Ala	Pro	Gly	Ser	Val	Gly	Pro	Pro	Asp	Glu	Ser	His	Pro	Gly	
		525				530					535					
agt	tct	gac	agc	tct	gcg	tct	cag	tcc	agt	gtg	aaa	aaa	gcc	agt	att	1802
Ser	Ser	Asp	Ser	Ser	Ala	Ser	Gln	Ser	Ser	Val	Lys	Lys	Ala	Ser	Ile	
540					545					550					555	
aaa	ata	ctg	aaa	aat	ttt	gat	gaa	gcg	ata	att	gtg	gat	gcg	gca	agt	1850
Lys	Ile	Leu	Lys	Asn	Phe	Asp	Glu	Ala	Ile	Ile	Val	Asp	Ala	Ala	Ser	
				560					565					570		
ctg	gat	cca	gaa	tct	tta	tat	caa	cgg	aca	tat	gcc	ggg	aag	atg	aca	1898
Leu	Asp	Pro	Glu	Ser	Leu	Tyr	Gln	Arg	Thr	Tyr	Ala	Gly	Lys	Met	Thr	
			575				580						585			
ttt	gga	aga	gtg	agt	gac	ttg	ggg	caa	ttc	atc	cgg	gaa	tct	gag	cct	1946
Phe	Gly	Arg	Val	Ser	Asp	Leu	Gly	Gln	Phe	Ile	Arg	Glu	Ser	Glu	Pro	
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<212> TYPE: PRT

<213> ORGANISM: Homo Sapien

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Lys Glu Gln Glu	Lys Thr Ser Asp Val Lys Ser Ile Lys Ala Ser Ile 35	40	45
Ser Val His Ser Pro Gln Lys Ser Thr Lys Asn His Ala Leu Leu Glu 50	55	60	
Ala Ala Gly Pro Ser His Val Ala Ile Asn Ala Ile Ser Ala Asn Met 65	70	75	80
Asp Ser Phe Ser Ser Arg Thr Ala Thr Leu Lys Lys Gln Pro Ser 85	90		95
His Met Glu Ala Ala His Phe Gly Asp Leu Gly Arg Ser Cys Leu Asp 100	105		110
Tyr Gln Thr Gln Glu Thr Lys Ser Ser Leu Ser Lys Thr Leu Glu Gln 115	120		125
Val Leu His Asp Thr Ile Val Leu Pro Tyr Phe Ile Gln Phe Met Glu 130	135		140
Leu Arg Arg Met Glu His Leu Val Lys Phe Trp Leu Glu Ala Glu Ser 145	150	155	160
Phe His Ser Thr Thr Trp Ser Arg Ile Arg Ala His Ser Leu Asn Thr 165	170		175
Met Lys Gln Ser Ser Leu Ala Glu Pro Val Ser Pro Ser Lys Lys His 180	185		190
Glu Thr Thr Ala Ser Phe Leu Thr Asp Ser Leu Asp Lys Arg Leu Glu 195	200		205
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Leu Asn Asn Arg Thr Asn Ser Thr Gln Asn His Leu Leu Leu Ser Gln 225	230	235	240
Glu Cys Asp Ser Ala His Ser Leu Arg Leu Glu Met Ala Arg Ala Gly 245	250		255
Thr His Gln Val Ser Met Glu Thr Gln Glu Ser Ser Ser Thr Leu Thr 260	265		270
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Tyr Ile Ser Pro Asp Ala Ala Lys Pro Ile Pro Ile Thr Glu Ala Met 305	310	315	320
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Gln Glu His Phe Ser Glu Phe Leu Arg Ser His His Phe Cys Lys Tyr 355	360		365
Gln Ile Glu Val Leu Thr Ser Gly Thr Val Tyr Leu Ala Asp Ile Leu 370	375		380
Phe Cys Glu Ser Ala Leu Phe Tyr Phe Ser Glu Tyr Met Glu Lys Glu 385	390	395	400
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gaagggtgaa cacagcatcc ctgctatctt cactcagacc ccagaaaacc cagggaacc      360
cgacagctcc actcccacca taacttatta ggagataagt cacattttat caacttgcca      420
tcgcgcctcc tatagattat acttcggtaa acccaatctg tataaattcc tttgtacttt      480
g                                                                                   481

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<210> SEQ ID NO 40
 <211> LENGTH: 390
 <212> TYPE: DNA
 <213> ORGANISM: Homo Sapien
 <300> PUBLICATION INFORMATION:
 <308> DATABASE ACCESSION NUMBER: GenBank AW874187
 <309> DATABASE ENTRY DATE: 2000-05-22

<400> SEQUENCE: 40

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ttttttttat tggactgtag gtttttatta aaacaaacat ttctcatagc tctaagcaaa      60
gcattagaat tcatcaagcg gactcacatc tttctctgc acagagaggg ctgaaaagg      120
agagaaagcc cttatgtat gtctagattt ggtaaagcga aggatttcag cgaatgagtc      180
actgaggcta tacacgtttg caaattgtaa ggcactggcg gccagagagc acagataaag      240
gacttttggg ggtcccccat tctgtccag caacctccca gctcacacct tagcttctac      300
caagaagggg tgaacacagc atccctgcta tcttactca gacccccaga agacacagga      360
aaccgcacag ctccactccc accataactt

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<210> SEQ ID NO 41
 <211> LENGTH: 43
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 41

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agcggataac aatttcacac agggagctag cttggaagat tgc      43

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<210> SEQ ID NO 42
 <211> LENGTH: 22

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 42
gtccaatata tgcaaacagt tg 22

<210> SEQ ID NO 43
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 43
agcggataac aatttcacac agg 23

<210> SEQ ID NO 44
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 44
actgagcctg ctgcataa 18

<210> SEQ ID NO 45
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 45
tctcaatcat gtgcattgag g 21

<210> SEQ ID NO 46
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 46
agcggataac aatttcacac agggatcaca cagccatcag cag 43

<210> SEQ ID NO 47
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: oligonucleotide primer

<400> SEQUENCE: 47
agcggataac aatttcacac agg 23

<210> SEQ ID NO 48
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Oligonucleotide primer

<400> SEQUENCE: 48
ctggcgccac gtggtcaa 18

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<210> SEQ ID NO 49
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 49
tttctctgca cagagagggc 20

<210> SEQ ID NO 50
<211> LENGTH: 44
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 50
agcggataac aatttcacac agggctgaaa tccttcgctt tacc 44

<210> SEQ ID NO 51
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 51
agcggataac aatttcacac agg 23

<210> SEQ ID NO 52
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 52
ctgaaaaggg agagaaag 18

<210> SEQ ID NO 53
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 53
tcccaaagtg ctggaattac 20

<210> SEQ ID NO 54
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 54
gtccaatata tgcaaacagt tg 22

<210> SEQ ID NO 55
<211> LENGTH: 20

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 55
cccacagcag ttaatccttc 20

<210> SEQ ID NO 56
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 56
gcgctcctgt cggtgcca 18

<210> SEQ ID NO 57
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 57
gcctgactgg tggggccc 18

<210> SEQ ID NO 58
<211> LENGTH: 15
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 58
catgcatgca cggtc 15

<210> SEQ ID NO 59
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 59
cagagagtac ccctcgaccg tgcatgcatg 30

<210> SEQ ID NO 60
<211> LENGTH: 15
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 60
catgcatgca cggtt 15

<210> SEQ ID NO 61
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

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<400> SEQUENCE: 61

gtacgtacgt gccaaactccc catgagagac

30

<210> SEQ ID NO 62

<211> LENGTH: 14

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 62

catgcatgca cggc

14

<210> SEQ ID NO 63

<211> LENGTH: 18

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 63

gcctgactgg tggggccc

18

<210> SEQ ID NO 64

<211> LENGTH: 26

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 64

gtgctgcagg tgtaaacttg taccag

26

<210> SEQ ID NO 65

<211> LENGTH: 28

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 65

cacggatccg gtagcagcgg tagagttg

28

<210> SEQ ID NO 66

<211> LENGTH: 19

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 66

actgggcatg tggagacag

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<210> SEQ ID NO 67

<211> LENGTH: 18

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 67

gcactttctt gccatgag

18

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<210> SEQ ID NO 68
<211> LENGTH: 14
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 68
tcagtcacga cggtt 14

<210> SEQ ID NO 69
<211> LENGTH: 14
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 69
cggataacaa ttctc 14

<210> SEQ ID NO 70
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 70
caatttcacg gctggatgca atctgggcta tgagatc 37

<210> SEQ ID NO 71
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 71
caatttcaca cagcggatgc ttcttttggc tctgact 37

<210> SEQ ID NO 72
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 72
tcagtcacga cggtggatgc caataaaagt gactctcagc 40

<210> SEQ ID NO 73
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 73
cggataacaa ttctggatgc actgggagca ttgaggc 37

<210> SEQ ID NO 74
<211> LENGTH: 38

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 74
tcagtcacga cgttggatga gcagatccct ggacaggc 38

<210> SEQ ID NO 75
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 75
cggataacaa tttcggatgg acaaaatacc tgtattcc 38

<210> SEQ ID NO 76
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 76
tcagtcacga cgttggatgc agagcagctc cgagtc 36

<210> SEQ ID NO 77
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 77
cagcggatgat cattggatgc aggaagctct gg 32

<210> SEQ ID NO 78
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 78
tcagtcacga cgttggatgc ccacatgcc cccactac 38

<210> SEQ ID NO 79
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 79
cggataacaa tttcggatgc ccgtcaggta ccacg 35

<210> SEQ ID NO 80
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

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<400> SEQUENCE: 80

tcagtcacga cgttggtatgc ccacagtga gcttcag 37

<210> SEQ ID NO 81

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 81

gctcatacct tgcaggatga cg 22

<210> SEQ ID NO 82

<211> LENGTH: 36

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 82

tcagtcacga cgttggtatga ccagctgttc gtgttc 36

<210> SEQ ID NO 83

<211> LENGTH: 34

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 83

tacatggagt tcggggatgc acacggcgac tctc 34

<210> SEQ ID NO 84

<211> LENGTH: 40

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 84

tcagtcacga cgttggtatgg ggaagagcag agatatacgt 40

<210> SEQ ID NO 85

<211> LENGTH: 29

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 85

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<210> SEQ ID NO 86

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 86

tgaagcactt gaaggatgag ggtgtctgcg 30

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<210> SEQ ID NO 87
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 87

cggataacaa tttcggatgc tgcgtgatga tgaaatcg 38

<210> SEQ ID NO 88
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 88

gatgaagctc ccaggatgcc agagggc 26

<210> SEQ ID NO 89
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 89

gccgccggtg taggatgctg ctggtgc 27

<210> SEQ ID NO 90
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide Template

<400> SEQUENCE: 90

cgcagggttt cctcgtcgca ctgggcattg g 31

<210> SEQ ID NO 91
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Biotinylated primer

<400> SEQUENCE: 91

tgcttatccc tgtagctacc ctgtcttggc cttgcagatc caa 43

<210> SEQ ID NO 92
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 92

agcggataac aatttcacac aggccatcac accgcggtac tg 42

<210> SEQ ID NO 93
<211> LENGTH: 44

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 93

cccagtcacg acgttgtaaa acgtcttggc cttgcagatc caag      44

<210> SEQ ID NO 94
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 94

agcgggataac aatttcacac aggccatcac accgcggtac tg      42

<210> SEQ ID NO 95
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 95

ctccagctgg gcaggagtgc      20

<210> SEQ ID NO 96
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 96

cacttcagtc gtcacct      17

<210> SEQ ID NO 97
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Biotinylated primer

<400> SEQUENCE: 97

cccagtcacg acgttgtaaa acg      23

<210> SEQ ID NO 98
<211> LENGTH: 100
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 98

cctttgagaa agggctctgc ttgagttgta gaaagaaccg ctgcaacaat ctgggctatg      60
agatcaataa agtcagagcc aaaagaagca gcaaaatgta      100

<210> SEQ ID NO 99
<211> LENGTH: 100
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 99

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cctttgagaa agggctctgc ttgagttgta gaaagaaccg ctgcaacaat ctgggctatg 60
agatcagtaa agtcagagcc aaaagaagca gcaaaatgta 100

<210> SEQ ID NO 100
<211> LENGTH: 100
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 100

gaattatattt tgtgtttcta aaactatggt tcccaataaa agtgactctc agcgagcctc 60
aatgctccca gtgctattca tgggcagctc tctgggctca 100

<210> SEQ ID NO 101
<211> LENGTH: 100
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 101

gaattatattt tgtgtttcta aaactatggt tcccaataaa agtgactctc agcaagcctc 60
aatgctccca gtgctattca tgggcagctc tctgggctca 100

<210> SEQ ID NO 102
<211> LENGTH: 84
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 102

taataggact acttctaato tgtaagagca gatccctgga caggcgagga atacaggtat 60
tttgtccttg aagtaacctt tcag 84

<210> SEQ ID NO 103
<211> LENGTH: 84
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 103

taataggact acttctaato tgtaagagca gatccctgga caggcaagga atacaggtat 60
tttgtccttg aagtaacctt tcag 84

<210> SEQ ID NO 104
<211> LENGTH: 100
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 104

ctcaccatgg gcatttgatt gcagagcagc tccgagtcog tccagagctt cctgcagtoa 60
atgatcacog ctgtgggcat cctgaggtc atgtctcgta 100

<210> SEQ ID NO 105
<211> LENGTH: 100
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 105

ctcaccatgg gcatttgatt gcagagcagc tccgagtcga tccagagctt cctgcagtoa 60
atgatcacog ctgtgggcat cctgaggtc atgtctcgta 100

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<210> SEQ ID NO 106
<211> LENGTH: 100
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 106

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agcaaggact cctgcaaggg ggacagtgga ggcccacatg ccaccacta ccagggcagc      60
tggtacctga cgggcacatcgt cagctggggc cagggctgcg                          100
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<210> SEQ ID NO 107
<211> LENGTH: 100
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 107

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agcaaggact cctgcaaggg ggacagtgga ggcccacatg ccaccacta ccggggcagc      60
tggtacctga cgggcacatcgt cagctggggc cagggctgcg                          100
```

<210> SEQ ID NO 108
<211> LENGTH: 100
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 108

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caataactct aatgcagcgg aagatgacct gccacagtg gagcttcagg gcgtggtgcc      60
ccggggcgctc aacctgcaag gtatgagcat accccccttc                          100
```

<210> SEQ ID NO 109
<211> LENGTH: 100
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 109

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caataactct aatgcagcgg aagatgacct gccacagtg gagcttcagg gcttggtgcc      60
ccggggcgctc aacctgcaag gtatgagcat accccccttc                          100
```

<210> SEQ ID NO 110
<211> LENGTH: 100
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 110

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ttgaagcttt gggctacgtg gatgaccagc tgttogtggt ctatgatcat gagagtgcg      60
gtgtggagcc ccgaactcca tgggtttcca gtagaatttc                          100
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<210> SEQ ID NO 111
<211> LENGTH: 100
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 111

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ttgaagcttt gggctacgtg gatgaccagc tgttogtggt ctatgatgat gagagtgcg      60
gtgtggagcc ccgaactcca tgggtttcca gtagaatttc                          100
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<210> SEQ ID NO 112
<211> LENGTH: 100

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<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 112
ggataacctt ggctgtacct cctggggaag agcagagata tacgtgccag gtggagcacc    60
caggcctgga tcagcccctc attgtgatct gggagccctc                            100

<210> SEQ ID NO 113
<211> LENGTH: 100
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 113
ggataacctt ggctgtacct cctggggaag agcagagata tacgtaccag gtggagcacc    60
caggcctgga tcagcccctc attgtgatct gggagccctc                            100

<210> SEQ ID NO 114
<211> LENGTH: 80
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 114
tgaagcactt gaaggagaag gtgtctgagg gagccgattt catcatcacg cagcttttct    60
ttgaggctga cacattcttc                                                    80

<210> SEQ ID NO 115
<211> LENGTH: 80
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 115
tgaagcactt gaaggagaag gtgtctgagg gagtcgattt catcatcacg cagcttttct    60
ttgaggctga cacattcttc                                                    80

<210> SEQ ID NO 116
<211> LENGTH: 80
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 116
tccagatgaa gctcccagaa tgccagaggc tgctccccgc gtggcccctg caccagcagc    60
tcctacaccg gcggcccctg                                                    80

<210> SEQ ID NO 117
<211> LENGTH: 80
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 117
tccagatgaa gctcccagaa tgccagaggc tgctccccgc gtggcccctg caccagcagc    60
tcctacaccg gcggcccctg                                                    80

<210> SEQ ID NO 118
<211> LENGTH: 48
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Hair pin structure

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<400> SEQUENCE: 118

cagagagtac cctcaaccg tgcattgcattg aaacatgcattg gcacgggtt

48

What is claimed is:

1. A method for determining whether a polymorphism correlates with susceptibility to morbidity, early mortality, or morbidity and early mortality, comprising;

identifying a polymorphism; and

determining the frequency of the polymorphism with increasing age in a healthy population.

2. The method of claim 1, wherein the polymorphism is identified by detecting the presence of target nucleic acids in a sample by a method, comprising the steps of:

- a) hybridizing a first oligonucleotide to the target nucleic acid;
- b) hybridizing a second oligonucleotide to an adjacent region of the target nucleic acid;
- c) ligating the hybridized oligonucleotides; and
- d) detecting hybridized first oligonucleotide by mass spectrometry as an indication of the presence of the target nucleic acid.

3. The method of claim 1, wherein the polymorphism is identified by detecting target nucleic acids in a sample by a method, comprising the steps of:

- a) hybridizing a first oligonucleotide to the target nucleic acid and hybridizing a second oligonucleotide to an adjacent region of the target nucleic acid;
- b) contacting the hybridized first and second oligonucleotides with a cleavage enzyme to form a cleavage product; and
- c) detecting the cleavage product by mass spectrometry as an indication of the presence of the target nucleic acid.

4. The method of claim 3 wherein the samples are from subjects in a healthy database.

5. The method of claim 1, wherein the polymorphism is identified by identifying target nucleic acids in a sample by primer oligo base extension (probe).

6. The method of 5, wherein primer oligo base extension, comprises:

- a) obtaining a nucleic acid molecule that contains a target nucleotide;
- b) optionally immobilizing the nucleic acid molecule onto a solid support, to produce an immobilized nucleic acid molecule;
- c) hybridizing the nucleic acid molecule with a primer oligonucleotide that is complementary to the nucleic acid molecule at a site adjacent to the target nucleotide;
- d) contacting the product of step c) with a composition comprising a dideoxynucleoside triphosphate or a 3'-deoxynucleoside triphosphate and a polymerase, so that only a dideoxynucleoside or 3'-deoxynucleoside

triphosphate that is complementary to the target nucleotide is extended onto the primer; and

e) detecting the extended primer, thereby identifying the target nucleotide.

7. The method of claim 6, wherein detection of the extended primer is effected by mass spectrometry, comprising:

ionizing and volatilizing the product of step d) ; and

detecting the extended primer by mass spectrometry, thereby identifying the target nucleotide.

8. The method of claim 7, wherein;

samples are presented to the mass spectrometer as arrays on chips; and

each sample occupies a volume that is about the size of the laser spot projected by the laser in a mass spectrometer used in matrix-assisted laser desorption/ionization (MALDI) spectrometry.

9. A method for determining a polymorphism that correlates with age, ethnicity or gender, comprising:

identifying a polymorphism; and

determining the frequency of the polymorphism with increasing age, with ethnicity or with gender in a healthy population.

10. The method of claim 1, wherein the polymorphism is identified by a method, comprising:

sorting the database according to a selected parameter to identify samples that match the selected parameter, wherein the database comprises:

datapoints representative of a plurality of healthy organisms from whom biological samples are obtained, wherein each

datapoint is associated with data representative of the organism type and other identifying information;

isolating a biopolymer from each identified sample;

optionally pooling each isolated biopolymer;

optionally amplifying the amount of biopolymer;

cleaving the pooled biopolymers to produce fragments thereof;

obtaining a mass spectrum of the resulting fragments and comparing the mass spectrum with a control mass spectrum to identify differences between the spectra and thereby identifying any polymorphisms; wherein:

the control mass spectrum is obtained from unsorted samples in the collection or samples sorted according to a different parameter.

11. The method of claim 10, wherein cleaving is effected by contacting the biopolymer with an enzyme.

12. The method of claim 11, wherein the enzyme is selected from the group consisting of nucleotide glycosylase, a nickase and a type IIS restriction enzyme.

13. The method of claim 10, wherein the biopolymer is a nucleic acid or a protein.

14. The method of claim 10, wherein the mass spectrometric format is selected from among Matrix-Assisted Laser Desorption/Ionization, Time-of-Flight (MALDI-TOF), Electrospray (ES), IR-MALDI, Ion Cyclotron Resonance (ICR), Fourier Transform and combinations thereof.

15. The method of claim 1, wherein the polymorphism is identified by a method, comprising:

obtaining samples of body tissue or fluid from a plurality of organisms;

isolating a biopolymer from each sample;

pooling each isolated biopolymer;

optionally amplifying the amount of biopolymer;

cleaving the pooled biopolymers to produce fragments thereof;

obtaining a mass spectrum of the resulting fragments;

comparing the frequency of each fragment to identify fragments present in amounts lower than the average frequency, thereby identifying any polymorphisms.

16. The method of claim 15, wherein cleaving is effected by contacting the biopolymer with an enzyme.

17. The method of claim 16, wherein the enzyme is selected from the group consisting of nucleotide glycosylase, a nickase and a type IIS restriction enzyme.

18. The method of claim 15, wherein the biopolymer is a nucleic acid or a protein.

19. The method of claim 15, wherein the mass spectrometric format is selected from among Matrix-Assisted Laser Desorption/Ionization, Time-of-Flight (MALDI-TOF), Electrospray (ES), IR-MALDI, Ion Cyclotron Resonance (ICR), Fourier Transform and combinations thereof.

20. The method of claim 15, wherein the samples are obtained from healthy subjects.

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